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Purine-Thiamine Anabolism During Cellular Morphogenesis in Myxococcus Xanthus

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PURINE-THIAMINE ANABOLISM DURING CELLULAR MORPHOGENESIS IN *MYXOCOCCUS XANTHUS*

BY

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122

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IN MYXOCOCCUS XANTHUS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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PURINE-THIAMINE ANABOLISM DURING CELLULAR MORPHOGENESIS IN *MYXOCOCCUS XANTHUS*

Abstract

WEN-CHERNG TSAI

Under the supervision of Associate Professor Carl A. Westby

Purine-thiamine anabolism during cellular morphogenesis in *Myxococcus xanthus* was investigated. One of the approaches was to test vegetative and myxospore (2.5 h and 8 h) extracts of *M. xanthus* CW-2 for their ability to synthesize the second *de novo* intermediate, 5'-phosphoribosylglycinamide, from beginning precursors either by way of phosphoribosyl-pyrophosphate amidotransferase (EC 2.4.2.14) or ribose-5-phosphate aminotransferase. Results indicate the presence of both the amido- and aminotransferases in both types of extracts, and both enzymes appear to be present at about the same level (per milligram of protein) in vegetative cells, myxospores (2.5 h and 8 h), and in a bacterial prototype, *Salmonella typhimurium*. The dose response of the CW-2 vegetative and myxospore amido- and aminotransferases towards AMP and GMP suggests that the allosteric structure of both enzymes is changed little by sporulation. Both the CW-2 amido- and aminotransferases were inhibited to varying degrees by a variety of purine nucleotides besides AMP, GMP, and 3':5' cyclic AMP.

The second approach in determining whether purine-thiamine anabolism functions in *M. xanthus* during cellular morphogenesis was to use whole cells. Whole cells (vegetative, myxospore and germinating

cells) were used to measure glycine, adenine, and guanine uptake and to test for the intracellular conversion of glycine-2-^{14}C to ^{14}C ATP and GTP. A thin-layer chromatographic-radioautographic method was employed to detect ^{14}C ATP and GTP. The finding that purine de novo partially repressed cells (vegetative, myxospore and germinating CW-1) take up glycine faster than fully repressed cells suggests that the purine de novo pathway can function throughout the life cycle of M. xanthus. The rate of glycine uptake is reduced about 50% during the first hour of glycerol-induced myxospore formation and germinating myxospores take up glycine, adenine, and guanine more rapidly than the other cell types. Purine uptake studies suggest the presence of interconversion and salvage routes in M. xanthus. The thin-layer chromatographic-radioautographic studies indicate that glycine-2-^{14}C is converted to ^{14}C ATP and GTP. A thiamine-like substance was also detected by this method and it may be related to myxospore induction. No evidence was found to support the presence of highly phosphorylated compounds or the magic spot during the life cycle of this organism.

A model for the thiamine or succinyl-CoA control of myxospore formation in M. xanthus is presented. This model proposes that a thiamine substance or succinyl-CoA may be a catabolite repressor or corepressor of myxospore (and fruiting-body) formation.

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I wish to dedicate this work to my parents, my wife and my son, whose love has been a perpetual source of inspiration during my academic career.

Finally, I dedicate this work to the memory of my brother, Dr. Kung-chow Tsai (1942-1975).

Wen-cherng Tsai

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Only three types of bacteria (excluding actinomycetes and blue green bacteria) are capable of undergoing extensive morphogenesis. These include the endospore-forming bacteria (endospore formation and germination), the nitrogen-fixing, cyst-forming azotobacters and the myxobacteria (myxospore formation and germination and fruiting). In the case of the family Myxobacteria, work by Hemphill and Zahler (52) indicates that added adenosine or a mixture of purines causes morphogenesis (myxospore formation and fruiting) in a histidine auxotroph. These findings and other evidence suggest that there exists a relationship between purine anabolism (de novo synthesis, salvage utilization and regulation) and morphogenesis in the myxobacteria. Justification for the origins of my study is based on this evidence. The overall aim of my investigation was to find out something about the biochemical mechanisms of myxospore induction and germination in the myxobacteria with special emphasis on the role of purine anabolism.

Myxococcus xanthus was chosen as the subject in my study because (i) it possesses one of the best known and studied life cycles among the myxobacteria; (ii) techniques for inducing large-scale, synchronous and rapid myxospore induction and germination are available; (iii) the organism is easy to grow in relatively simple media with a short generation time; and (iv) active enzymes are extractable from myxospores as well as vegetative cells.

Purine nucleotides participate as fully one-half of the total subunits of the biopolymers, DNA and RNA. Pyrimidine nucleotides

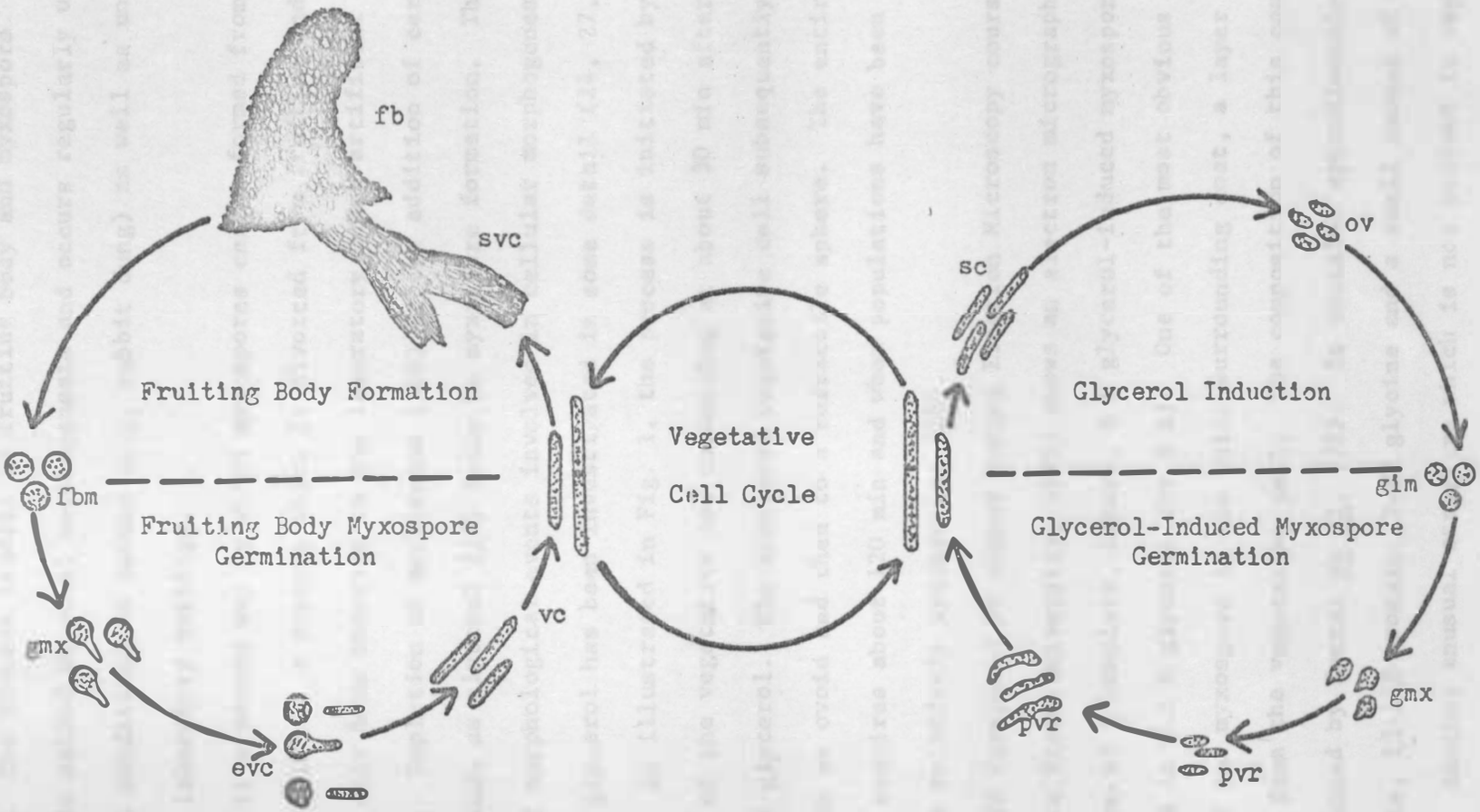
comprise the other one-half. Purine nucleotides also participate separately as monomers in many aspects of intermediary metabolism as substrates (e.g. ATP for histidine synthesis), cofactors (e.g. NAD for many reactions catalyzed by dehydrogenases) or regulatory molecules (e.g. cyclic AMP for regulating cell division) (53). Purine ribonucleotides, one physiologically active form of purines, can be synthesized by various microorganisms de novo from simple biochemical precursors such as ribose-5-phosphate, L-glutamine and glycine. Deoxyribonucleotides are only formed from preformed ribonucleotides. Most microorganisms also have the capacity to utilize preformed external purines in which case the aglycones are actively transported into the cell and are simultaneously phosphoribosylated to form ribonucleotides. In this situation, de novo synthesis is quickly terminated by enzyme inhibition and/or gene repression to prevent wasteful endogenous synthesis of the same materials. The de novo pathway is also involved in the biosynthesis of the vitamin thiamine and the amino acid histidine. Regulation of the purine de novo pathway, therefore affects the synthesis of thiamine and histidine as well as purine nucleotides.

REVIEW OF LITERATURE

The Life Cycle and Morphology of M. xanthus

The life cycle of M. xanthus is summarized and illustrated in Fig. 1. Vegetative cells of M. xanthus are rod-shaped and approximately 0.75 to 5.2 μm wide by 3.0 to 7.5 μm in length. Electron micrographs of thin sections of vegetative cells reveal a typical procaryotic nucleus and cytoplasm surrounded by a multilayered wall-membrane complex characteristic of Gram-negative bacteria (154). The vegetative rods have a developmental option. When placed in an enriched medium such as 1-2% casitone with magnesium sulfate, they will grow for an extended period of time with a generation time of approximately 4.5 h. Under certain nutritionally limiting conditions, however, a population of vegetative cells on a solid substrate tends to fruit, first by swarming and then by migrating in a coordinated fashion toward specific centers of aggregation eventually to form macroscopic fruiting bodies (23, 24, 27, 31, 137, 154, 158). Within the fruiting bodies, the individual cells undergo a series of morphogenetic events to form myxospores. Dworkin and Voelz (31), by using time-lapse photography coupled to phase-contrast microscopy, showed that myxospore formation involves an initial shortening and thickening of the entire vegetative cell with a subsequent rounding and increase of refractility. Each fruiting body may consist of 40,000 or more myxospore (158) piled orderly and in a structured way upon hardened slime polysaccharide and

Fig. 1. Life cycle of Myxococcus xanthus (137). Abbreviations: fb, fruiting body; fbm, fruiting-body myxospores; gmx, germinating myxospore; evc, emerging vegetative cells; vc, vegetative cells; svc, swarming vegetative cells; sc, shortening vegetative cells; ov, ovoids; gim, glycerol-induced myxospores; pvr, pleomorphic vegetative rods.

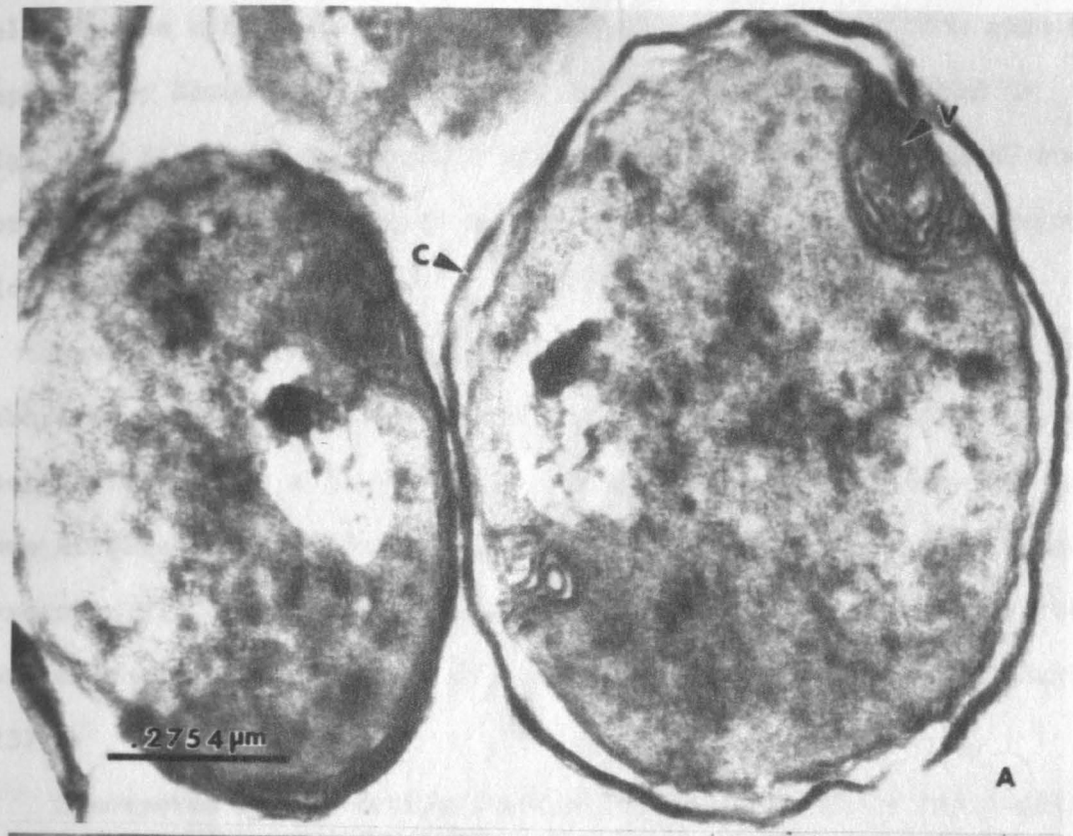

fb
svc
Fruiting Body Formation
fbm
Fruiting Body Myxospore
Germination
gmx
evc
vc
Vegetative
Cell Cycle
sc
ov
Glycerol Induction
gim
Glycerol-Induced Myxospore
Germination
gmx
pvr
pvr

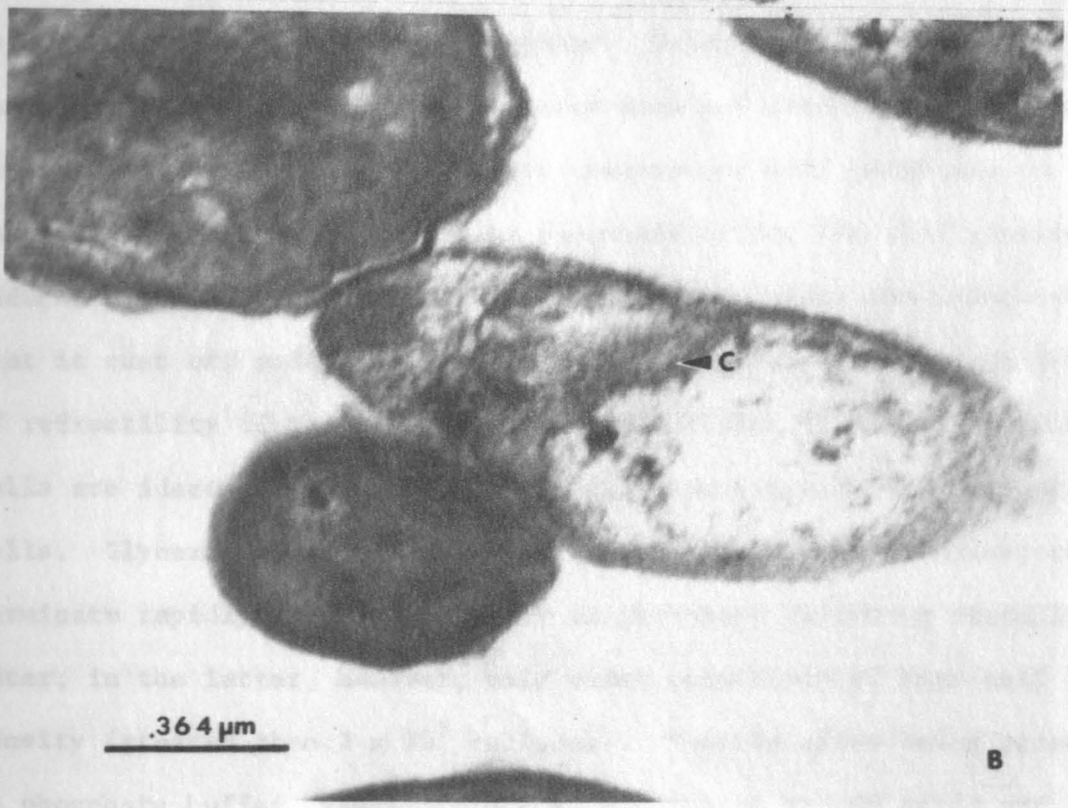
dead cells. The process leading to fruiting body and myxospore formation is called colonial morphogenesis and occurs regularly under appropriate conditions in nature (e.g. rabbit dung) as well as under prescribed laboratory settings.

There is a second way by which myxospores can be formed from vegetative cells in a process which is divorced from fruiting and which has so far only been observed in the laboratory under artificial conditions. Depletion of methionine (160) or the addition of certain compounds such as glycerol (28) leads to myxospore formation. The sequence of morphological events involved in cellular morphogenesis induced by glycerol has been investigated in some detail (24, 27, 28, 128, 137). As illustrated in Fig. 1, the process is initiated by a shortening of the vegetative rod commencing at about 30 min after the addition of glycerol. The shortened vegetative cell subsequently is converted to an ovoid and then to a refractile sphere. The entire conversion requires about 120 min and when populations have been examined is relatively synchronous (28).

Fig. 2A (prepared by author during Electron Microscopy course at South Dakota State University, 1975) shows an electron micrograph of a thin section of a complete, mature, 8 h glycerol-induced myxospore (maintained in 0.5 M glycerol for 8 h). One of the most obvious features of the myxospore is the thick surrounding coat, a layer which is missing from the vegetative cell. The composition of this coat has been determined by Kottel et al. (72). It contains approximately 70% carbohydrate, 13-14% protein, 7-8% glycine and a small amount of organic phosphate. Another unusual structure which is not present in vegetative

Fig. 2. Thin sections of 8 h (A) and germinating 8 h (B) glycerol-induced myxospores. The myxospore coat (C) and vesicle (V) are indicated. Magnification: (A) x90,000, (B) x75,000. Electron micrograph prepared by author during Electron Microscopy course at South Dakota State University, 1975.


V
C
.2754 µm
A


C
.364 µm
B

cells is the cytoplasmic membranous vesicle. These vesicles were first reported by Bacon and Eiserling (2) and are also demonstrated in Fig. 2A. Although the kinetics of formation of these vesicles have not yet been determined, they appear to function in myxospore germination (24, 137).

Myxospores formed naturally during fruiting or artificially in the laboratory are resistant to desiccation, ultraviolet irradiation, sonic oscillation and to a lesser extent, heat (142). There are, however, some differences in terms of size (2.0 μm in diameter for fruiting-body myxospore, 1.2 μm for glycerol-induced myxospore) and time required for maturation (6 to 9 days for the former, and 2 h for the latter) (137).

Myxospores formed during fruiting or in its absence can readily germinate under appropriate conditions. Unlike bacterial endospore germination, germination in myxospores does not seem to be preceded by activation (24, 143). Fruiting-body myxospores will germinate in complete growth medium but not in phosphate buffer (as will glycerol-induced myxospores) (158). During their germination, the myxospore coat is cast off and this is followed by a gradual elongation and loss of refractility of the germinating cell (see Fig. 1) (154). Germinated cells are identical physiologically and morphologically to vegetative cells. Glycerol-induced myxospores, unlike fruiting-body myxospores, germinate rapidly and synchronously in phosphate buffer or distilled water; in the latter, however, only under conditions of high cell density (greater than 2×10^9 cells/ml). Shortly after being placed in phosphate buffer, glycerol-induced myxospores become ovoid and

vegetative cells emerge through one end of the coat (as shown on Fig. 2B). The coat is believed to be maintained as part of the emerging vegetative cell and, eventually, either fuses with the cell wall or slowly disintegrates (137). As shown in Fig. 1, the surface of the enlarged vegetative cell becomes distorted and bulges along the length of cell and then subsequently assumes the normal appearance of a typical vegetative cell.

Nutritional Control of Morphogenesis in M. xanthus

The observed onset of fruiting, myxospore induction and germination in M. xanthus in response to fluctuations in nutritional and physical circumstances has been repeatedly observed during the past decade. These observations suggest that the triggering of these various types of morphogenesis is subject to nutritional control. It has been shown, in this regard, that there is an intimate relationship between fruiting and nutritional conditions (14, 22, 52, 124). As indicated in Table 1, the absence of any one of a variety of amino acids such as phenylalanine, tryptophan, glycine, isoleucine, leucine or valine (in a chemically defined medium) stimulates fruiting (52). On the other hand, high concentrations of the above amino acids (52) or the polyamine, spermidine (124), prevent fruiting body formation. Table 1 shows that fruiting bodies in general are formed when cells are grown on relatively poor nutrient media and, conversely, prevented when cells are grown on rich media. Fruiting may also be enhanced by the presence of purine nucleotides (e.g. cyclic AMP or ADP) in poor media (14). Thus, it

Table 1. Compounds that stimulate or inhibit fruiting, myxospore induction and germination by their presence or absence.

	Presence or Absence of		Reference
Fruiting	Stimulatory effect	Presence of:	
		1. low concentrations of peptone	(109)
		2. cyclic AMP, ADP, threonine or D,L-diaminopimelic acid plus cyclic AMP.	(14)
		3. low concentrations of casitone, isoleucine, lysine or homoserine	(14)
		Absence of:	
		1. phenylalanine, isoleucine, leucine methionine, valine, glycine, proline, tryptophan or lysine.	(52)
	Inhibitory effect	Presence of:	
		1. ethionine, norleucine, lysine or casitone	(52)
		2. phenylalanine, tryptophan, methionine or spermidine.	(52)
Myxospore induction	Stimulatory effect	Presence of:	
		1. ethionine (but no methionine), isoleucine plus/or threonine, or putrescine.	(169)
		2. glycerol, n-propanol, or certain other related compounds.	(39)
		Absence of:	
		1. methionine.	(169)
	Inhibitory effect	Presence of:	
		1. spermidine.	(169)
Fruiting-body myxospore germination	Stimulatory effect	Presence of:	
		1. complete CT medium.	(31)
Non-fruiting-body myxospore germination	Stimulatory effect	Presence of:	
		1. complete CT medium.	(119)
		2. protein lysate, glycine, alanine, valine, aspartic acid or glutamic acid.	(119)
		3. $HPO_4^{=}$, Mg^{++}, Ca^{++}, NH_4^{+}	(119)
		4. distilled water (high cell density required)	(119)

appears that the deficiency of certain amino acids and related compounds triggers fruiting as likewise does the excess of others. The situation, however, may be somewhat more complicated than just this. For example, Rosenberg et al. (124) found that threonine completely reverses the inhibitory action of methionine and isoleucine. Considering such complications it may still be reasonable, however, to suggest that fruiting is a direct response to the disappearance of critical elements of nutrition that are necessary to sustain vegetative growth.

Myxospore induction, like fruiting, seems also to be under nutritional control. As indicated in Table 1, the presence of high concentrations of glycerol or other compounds containing primary or secondary alcoholic groups in a complex liquid medium induces myxospore formation (fruiting body formation does not take place) (30). Sadler and Dworkin (127) found that the amount of inducer incorporated by cells during their conversion to myxospores is only a very small fraction of the overall. They suggested that inducers may alter a membrane-DNA relationship initiating the read-off of a genome associated with myxospore formation. Witkin and Rosenberg (160), however, suggested that there is a competition between glycerol and spermidine, an inhibitor of myxospore formation. They studied myxospore induction resulting from amino acid over-abundance or starvation and found that both isoleucine and threonine in relatively high concentration induced myxospore formation. Media lacking methionine also was found to bring about the formation of myxospores from vegetative cells (160). Putrescine, a polyamine, was observed to stimulate myxospore formation

whereas another polyamine, spermidine, prevented the cellular morphogenesis (160). The stimulation of myxospore formation by either over-abundance or deficiency of certain amino acids seems to be parallel to what occurs in fruiting body formation. There are no clues so far as to the relationship, if any, between myxospore induction and fruiting body formation.

Myxospore germination, like myxospore induction, is also probably brought about by changes in the concentration of extra-cellular nutrients. As indicated in Table 1, fruiting-body myxospores readily germinate when placed on an appropriate medium containing casitone (2%), $MgSO_4$ (0.1%), and K_2HPO_4-KH_2PO_4 (0.01 M, pH 7.2) (110). Glycerol-induced myxospores will germinate in the presence of protein hydrolysates or various individual amino acids such as glycine, alanine, valine, aspartic acid, and glutamic acid. Inorganic ions or a phosphorylated substance secreted by the myxospore also readily induced germination of glycerol-induced myxospores (110). Recent evidence (26) has indicated that inorganic phosphate or a related phosphorylated compound such as cyclic AMP may be the agent that induces germination. The finding that glycerol-induced myxospores will germinate in distilled water if the cell population is high (24, 110) may be of particular interest in explaining the mechanism of germination. Dworkin (26) suggested that a sufficiently high cell population is necessary in order to guarantee a swarm for maximizing nutrient collection.

Biochemical Events During Myxospore Formation and Its Germination

Protein synthesis

Protein synthesis is required for glycerol-induced myxospore formation (127) and germination (62, 110). During the first two hours of glycerol-induced myxospore formation there occurs a 35%, non-linear increase in total protein. When refractile myxospores appear, protein synthesis levels off. The uptake of ^{14}C-leucine is linear throughout the conversion, indicating that rapid turnover of protein and recycling of leucine is taking place (127). Some enzymes (see Table 2), such as isocitrate lyase (EC 4.1.3.1), malate synthesis (EC 4.1.3.2), alkaline phosphatase (EC 3.1.3.1), fructose-1,6-diphosphatase (EC 3.1.3.11) and proteolytic enzymes increase during glycerol-induced myxospore formation. These appear to be myxospore specific enzymes. Their increase during myxospore induction may, at least in part, account for the increase in total protein during induction. The plateau in net protein synthesis after the completion of myxospore formation may be caused by inactivation and degradation of some of the above enzymes such as isocitrate lyase (104).

Protein synthesis is also necessary for myxospore germination since the addition of rifampin, an inhibitor of protein synthesis, interferes with germination (62). The increase of certain enzymes such as alkaline phosphatase during germination (26) may be the result of new protein synthesis in which case the increase should be inhibited by rifampin.

Table 2. Enzyme status during myxospore formation of M. xanthus.

Status During Myxospore Induction	Name of Enzyme
Activity unchanged	1. glycine dehydrogenase (73).
	2. alanine dehydrogenase (73).
	3. glutamate dehydrogenase (73).
	4. glutamic glyoxylate aminotransferase (73).
	5. alanine glyoxylate aminotransferase (73).
	6. alanine aminotransferase (43).
	7. aspartate aminotransferase (43).
	8. enzymes of glycolytic pathway (156).
	9. enzymes of TCA cycle (except isocitrate dehydrogenase) (156).
	10. enzymes of electron transport system (29).
Activity increases	1. isocitrate dehydrogenase (156).
	2. isocitrate lyase (7, 103).
	3. malate synthase (7, 103).
	4. enzymes for synthesizing UDP-N-acetyl-galactose-amine (158).
	5. alkaline phosphatase (26).
	6. disulfide reductase (44).
	7. intracellular proteolytic enzymes (104).
Activity decreases	1. aspartokinase (124).

DNA synthesis

Rosenberg et al. (123) reported that exponentially growing vegetative cells contain two chromosomes that replicate sequentially during 80% of the generation cycle. During glycerol induced myxospore formation, the DNA content of cells increases about 20% above that of the vegetative cell (123, 127). It has been calculated (167) that if either of the two sequentially replicating bacterial chromosomes finishes its round of replication, but no new rounds are initiated, the net increase of DNA in a cell population would be 20%. Chromosome sequential replication in M. xanthus was confirmed by populational analyses of grain count distribution after pulsing induced myxospores with tritiated thymidine (168).

Zusman and Rosenberg (167) studied DNA synthesis during myxospore germination in a complex medium and found that no DNA synthesis occurred for 3.5- to 4-h following the onset of germination. After this lag, however, synchronous chromosome replication did occur. They concluded that two successive divisions took place after the 3.5-4.0 h delay, restoring the normal chromosomal complement to the emerging vegetative cell.

RNA synthesis

Bacon and Rosenberg (3) measured RNA synthesis during myxospore induction in M. xanthus and found that, after glycerol induction, net RNA synthesis immediately terminated. However, extensive turnover did still take place, including RNA made both before and after induction (3, 127). The nature of the RNA synthesized during myxospore induction

has been characterized by fractionating the RNA (3) and by using a DNA-RNA hybridization technique (102). All classes of RNA (messenger RNA, ribosomal RNA and transfer RNA) are synthesized during myxospore formation and although ribosomal RNA (rRNA) synthesized during myxospore formation is indistinguishable from that made during vegetative growth, certain messenger RNA (mRNA) species are synthesized only during vegetative growth. Ramsey and Dworkin (111) reported that existence of a stable mRNA synthesized 4- to 5-h after initiation of myxospore formation. Other researchers (3, 33), however, have not been able to confirm the presence of such a stable mRNA. Whether stable mRNA is present in myxospores still remains in question.

As is the case during endospore germination (161), RNA synthesis is likewise required during myxospore germination in M. xanthus (61, 62). Juengst and Dworkin (62) found that RNA was synthesized during the first 25- to 35-min of germination.

Metabolic patterns

A chemically defined medium containing 17 amino acids but lacking any readily catalyzable carbon source (e.g. glucose) was found by Dworkin (21) to support the growth of M. xanthus. This indicated that amino acids apparently serve as sources of energy as well as providing carbon and nitrogen and that the oxidative degradation of amino acids should occur in this organism. In addition, the multiplicity of amino acids required in the defined medium suggested a limited capacity for the synthesis of amino acids. As indicated in Table 2, the enzymes of amino acid metabolism such as glycine dehydrogenase (EC 1.4.1.5),

alanine dehydrogenase (EC 1.4.1.1), glutamate dehydrogenase (EC 1.4.1.2), glutamic glyoxylate aminotransferase (EC 2.6.1.4), alanine glyoxylate aminotransferase (EC 2.6.1.12) (73), alanine aminotransferase (EC 2.6.1.2), and aspartate aminotransferases (EC 2.6.1.1) (43) occur in approximately equal amounts in both vegetative cells and glycerol-induced myxospores.

The intermediary metabolism of M. xanthus during glycerol-induced myxospore formation has been studied extensively. Watson and Dworkin (156), for example, found that there were no significant differences in the activities of enzymes involved in the glycolytic and tricarboxylic acid (TCA) pathways when cell-free extracts from vegetative cells were compared with glycerol-induced myxospores (see Table 2). Hexokinase (EC 2.7.1.1) and pyruvate kinase (EC 2.4.1.40), the first and the last enzyme, respectively, of the glycolytic pathway were found, however, to be absent in both vegetative cells and glycerol-induced myxospores. In order to account for the absence of these two enzymes, Watson and Dworkin (156) suggested that the principal function of the glycolytic enzymes in M. xanthus is gluconeogenic. Since all of the TCA emzymes are present in vegetative cells and myxospores, it was suggested that amino acids are converted to TCA intermediates and then to gluconeogenic intermediates and eventually to glucose and polysaccharides. This would provide an explanation for the production of large amounts of extracellular slime during vegetative growth on amino acids.

Dworkin and Niederpruem (29) examined the terminal electron transport system in M. xanthus and found that there were no quantitative differences in the enzymes of vegetative cells compared to

fruiting-body myxospores (see Table 2), although qualitative differences between the two types of cells may have existed. The finding of greater cytochrome reductase and cytochrome oxidase activity in fruiting-body myxospores compared to vegetative cells was believed to be caused by cultivation conditions (solid versus liquid medium) or the state of aerobiosis (solid versus liquid medium) rather than because of basic differences between the two types of cells (23). In spite of the fact that fruiting-body myxospores possess all enzymes of the electron transport system, they, like bacterial endospores, display no measurable endogenous respiration when 2% casitone, the usual substrate, is added. In contrast to fruiting-body myxospore, glycerol-induced myxospores respire when the 2% casitone is added. The rate, however, is slower than that for vegetative cells (143).

It was mentioned earlier that the observed increase of protein synthesis during cellular morphogenesis could have resulted in an increase of some myxospore specific or myxospore enriched enzyme-proteins. This would account for the shifts in metabolic activities that occur during cellular morphogenesis such as the observed increase of the glyoxylate cycle that occurs during myxospore induction (7, 103). Because chloramphenical prevented an increase in activity of two of the glyoxylate cycle enzymes (isocitrate lyase and malate synthase) during myxospore formation, Bland et al. (7) concluded that both enzymes are synthesized de novo, rather than being activated from a preexisting inactive state. The shift to glyoxylate cycle enzymes during myxospore formation may reflect a myxospore dependence upon the

metabolism of two-carbon compounds. The glyoxylate formed as a result of isocitrate lyase activity is primarily converted either to cell coat glycine via glycine dehydrogenase and/or transaminase (73) or to malate to reenter the glyoxylate cycle.

The enzymes responsible for synthesis of UDP-N-acetylgalactosamine from fructose-6-phosphate (e.g. fructose-1,6-diphosphatase and UDP-N-acetyl-galactosamine biosynthetic enzymes) increase during myxospore formation (158). Shortly before completion of the myxospore coat, these enzymes and the glyoxylate cycle enzymes (103, 105) begin to decrease to the level found in vegetative cells.

Dworkin (26) examined the kinetics of alkaline phosphatase formation during myxospore formation and noted that the enzyme's activity increases about tenfold after 3 h of glycerol induction and then gradually levels off. He suggested that the rise of alkaline phosphatase is responsible for the subsequent release of phosphate from CMP in the intracellular nucleotide pool.

Another enzyme that increases during glycerol-induced myxospore formation is disulfide reductase (44). Its increase indicates that disulfide is required in larger quantities during myxospore induction than during vegetative growth.

Thus far, there has been discovered only one enzyme, aspartokinase (EC 2.7.2.4) that drops in level during myxospore formation (124). Its significance and possible relationship to myxospore formation will be discussed later.

Possible Mechanisms of Morphogenesis in M. xanthus

Fruiting body formation

While investigating the mechanisms of fruiting body formation in M. xanthus, Rosenberg et al. (124) found a relationship between reduced aspartokinase activity and fruiting; (i) aspartokinase activity was found to be repressed and feed-back inhibited by lysine and threonine, dual inducers of fruiting body formation and the enzyme's activity was stimulated by isoleucine and methionine, dual inhibitors of fruiting body formation; and (ii) a mutant which was capable of forming fruiting bodies in a relatively high concentration of casitone (usually reduced levels of casitone are required for fruiting) exhibited a decreased aspartokinase activity. They proposed a "DAP hypothesis", i.e. that a decrease in aspartokinase activity results in diaminopimelic acid (DAP) starvation, blockage of cell wall synthesis and subsequent stimulation of fruiting.

Recently, Campos and Zusman (14) reported evidence implicating cyclic AMP and ADP, in fruiting body formation. They developed an assay system to measure fruiting body formation and found that both cyclic AMP and ADP greatly stimulated the number of fruiting bodies produced on the assay medium. They also investigated the effect of amino acids on the nucleotide stimulation of fruiting body formation. They found that methionine and isoleucine completely blocked the action of cyclic AMP and ADP and that threonine or DAP synergistically enhanced the fruiting body formation exerted by both nucleotides. This and the

finding that the inhibition of fruiting by methionine or isoleucine cannot be overcome by the addition of cyclic AMP and ADP suggests that there are at least two regulatory controls on fruiting body formation.

Fruiting body formation can be induced by the depletion of certain amino acids from the medium. Dworkin (24) therefore, suggested that a chemotactic agent is produced after release of catabolite repression by depletion of a specific nutrient. He also proposed that the formation of the fruiting body requires communication and cooperation among cells and that such behavior is under control of the chemotactic agent. There is no evidence to support the presence of such substances during fruiting body formation, however, although some exogenously supplied nucleotides or amino acids are able to enhance fruiting.

Myxospore induction

Myxospores embedded within the fruiting body are dormant cells, i.e. they are not dividing; their intermediary metabolism has largely ceased and they are more resistant to the environment than the vegetative cells. Dworkin (24), thus, suggested that the fruiting body proper may be responsible for providing the proper environment to trigger the formation of myxospores. Hanson and Dworkin (45) hypothesized that a state of desiccation is created by fruiting that induces the fruiting cells to become dormant myxospores. Because high cell density appears to be required for fruiting in M. xanthus, Dworkin (26) has suggested that the accumulation of certain metabolic intermediates triggers the vegetative cells to form myxospores. How vegetative cells

interact among themselves to become an aggregate in order to provide the proper milieu for myxospores formation still remains vague.

The discovery that it was possible to short-circuit fruiting body formation and to induce myxospore formation directly by glycerol exposure (28) made investigation of the mechanism of myxospore formation amenable to laboratory analysis. Sadler and Dworkin (127) suggested that induction of myxospore formation by glycerol may be the result of the inducer, acting on the cell membrane and changing its physical properties in such a way as to initiate the developmental process. Until now, however, there is still no confirmatory evidence to support this theory nor elaboration on the details of this model.

Hanson and Dworkin (45) examined the levels of endogenous nucleotides during myxospore formation and discovered that there was a clear tendency for the levels of the endogenous nucleotide pools, with some exception, to increase during the process. In addition, no extracellular nucleotide disphosphate or triphosphate compounds were detected during myxospore induction. It was considered possible that the nucleotide pools could have been formed either de novo (see later section for details) or by a salvage route (see later section for details). Indirect evidence for the involvement of purine de novo synthesis in myxospore morphogenesis comes from the findings of Hemphill and Zahler (52), who examined a histidine-required auxotrophic mutant of M. xanthus (strain FBa) and observed that excess external purines stimulated fruiting body and myxospore formation. Since the histidine and purine biosynthetic pathways share common steps (to be

discussed later), the result of Hemphill and Zahler suggests that the regulation of purine de novo synthesis may be involved in controlling fruiting body and myxospore formation.

Elmerich and Aubert (32) found that Bacillus megaterium mutants blocked early in the purine de novo pathway (between PP-ribose-P and AIR; see later section) continue to sporulate even when nutrients are available that prevent sporulation of later blocked purine mutants. They concluded that a mutation in a step between PP-ribose-P and AIR brings about derepression of sporulation. The relationship between this and myxospore formation is unclear.

Recently, Rhaese and coworkers (118, 119, 120, 121, 122) found that highly phosphorylated nucleotides (HPN), such as HPN I (adenosine tetraphosphate) and HPN II (adenosine pentaphosphate), and the magic spot compound (MS) (guanosine tetra- and pentaphosphates) accumulate during the sporulation of B. subtilis. Since the inhibition of sporulation by excess glucose is accompanied by an inhibition of HPN production, they suggested that these highly phosphorylated nucleotides may be involved in the regulation of sporulation. By using mutants unable to synthesize MS, they also found that MS is apparently not needed for sporulation (120). Myxospores have not yet been examined for HPN.

Myxospore germination

The regulatory mechanisms of myxospore germination of M. xanthus are still unknown. The finding, however, that inorganic phosphate and other unknown substances (germination factor) secreted by myxospores will initiate the germination in distilled water (110) gives some clues

as to the mechanism of germination. The unknown substances have not been analyzed but they may be purine nucleotides, e.g. cyclic AMP or other phosphorylated metabolites. Recently, Hanson and Dworkin (45) found that, during myxospore germination, there occurs excretion of substantial amounts of AMP. This indicates that the synthesis of purine nucleotides (from de novo or salvage route) may control the myxospore germination.

The De Novo Synthesis of Purine Nucleotides and Gene Relationships

The first important clues to the biosynthetic origins of the purine bases came from the experiments of Buchanan and his coworkers (12, 13, 77, 139, 140, 141). They fed various suspected precursors labelled with ^{14}C or ^{15}N to live birds and determined the sites of incorporation of the labeled atoms into the purine ring. It was shown in this way that nitrogen atoms 3 and 9 arise from the amide group of glutamine, nitrogen 1 from aspartate, and nitrogen 7 from glycine. Carbon atoms 4 and 5 were found to be derived from glycine, indicating that the backbone of the glycine molecule is directly incorporated into the purine ring. Carbon atoms 2 and 8 are furnished by formate and carbon 6 by CO_2. The other naturally occurring purines were thereafter shown to be synthesized in a similar fashion (10). In Fig. 3, the origins of the nitrogens and carbons of the purine, inosinic acid (IMP) are depicted schematically.

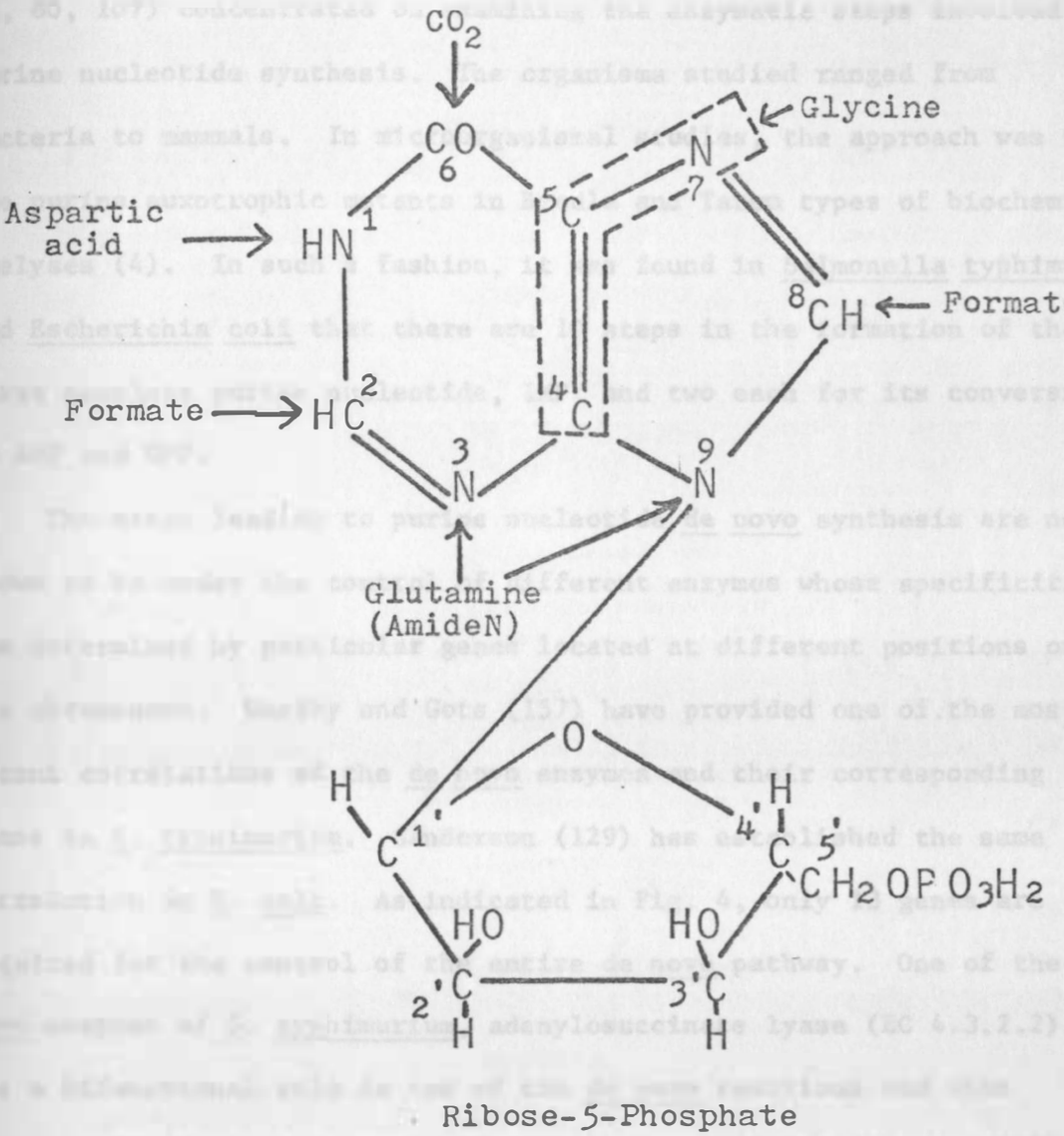


Fig. 3. The biological origin of the carbon and nitrogen atoms of the purine, inosinic acid.

After this approach had been fully exploited, researchers (11, 36, 38, 80, 107) concentrated on examining the enzymatic steps involved in purine nucleotide synthesis. The organisms studied ranged from bacteria to mammals. In microorganismal studies, the approach was to use purine auxotrophic mutants in Beadle and Tatum types of biochemical analyses (4). In such a fashion, it was found in Salmonella typhimurium and Escherichia coli that there are 10 steps in the formation of the first complete purine nucleotide, IMP, and two each for its conversion to AMP and GMP.

The steps leading to purine nucleotide de novo synthesis are now known to be under the control of different enzymes whose specificities are determined by particular genes located at different positions on the chromosome. Westby and Gots (157) have provided one of the most recent correlations of the de novo enzymes and their corresponding genes in S. typhimurium. Sanderson (129) has established the same correlation in E. coli. As indicated in Fig. 4, only 13 genes are required for the control of the entire de novo pathway. One of the de novo enzymes of S. typhimurium, adenylosuccinate lyase (EC 4.3.2.2), has a bifunctional role in two of the de novo reactions and this accounts for their being only 13 genes to control the 14 steps (41). The genes pur-D, pur-H and pur-J are located at the same map position on the S. typhimurium chromosome (Fig. 4) and are believed to act as a functioning operon. The same is also true for gua-A and gua-B. The remaining genes are scattered among different locations on the chromosome.

Enzyme	Reaction	Gene	Map position[a]
	PR-1'-pyrophosphate (PR-ribose-P)		
1. PP-ribose-P amidotransferase (EC 2.4.2.14)	↓	pur-F	73
	Phosphoribosylamine (PRA)		
2. GAR synthetase (EC 6.3.1.3)	↓	pur-D	129
	PR-glycinamide (GAR)		
3. Formyl-GAR transferase (EC 2.1.2.2)	↓	none	---
	PR-formyglycinamide (Formyl-GAR)		
4. Formyl-GAR amidotransferase (EC 6.3.5.3)	↓	pur-G	80
	PR-formylglycinamidine (Formyl-GAM)		
5. AIR synthetase (EC 6.3.3.1)	↓	pur-I	79
	PR-aminoimidazole (AIR)		
6. AIR carboxylase (EC 4.1.1.21)	↓	pur-E	19
	PR-aminoimidazolecarboxylate (C-AIR)		
7. S AICAR synthetase (EC 6.3.2.6)	↓	pur-C	79
	PR-aminoimidazolesuccino-carboxamide (S-AICAR)		
8. Adenylosuccinate lyase (EC 4.3.2.2)	↓	pur-B	43
	PR-aminoimidazolecarboxamide (AICAR)		
9. AICAR formyltransferase (EC 2.1.2.3)	↓	pur-H	129
	PR-formamidoimidazole-carboxamide (F-AICAR)		
10. IMP cyclohydrolase (EC 3.5.4.10)	↓	pur-J	129
	Inosine-5'-phosphate (IMP)		
11. SAMP synthetase (EC 6.3.4.4)	↓	pur-A	136
	Adenylosuccinate (SAMP)		
12. Adenylosuccinate lyase (EC 4.3.2.2)	↓	pur-B	43
	Adenosine-5'-phosphate (AMP)		
13. IMP dehydrogenase (EC 1.2.2.14)	↓	gua-B	79
	Xanthine-5'-phosphate (XMP)		
14. XMP aminase (EC 6.3.5.2)	↓	gua-A	79
	Guanosine-5'-phosphate (GMP)		

Figure 4. Gene-enzyme relationships in the purine pathway of S. typhimurium (129,157).

[a] Map position: numbers refer to time scale on the map published by Sanderson (129). PR = 5'phosphoribosyl.

The first indication of the presence of purine nucleotide de novo synthesis in M. xanthus came from the nutritional studies of Dworkin (21), who devised a defined synthetic medium lacking any purine that fully supported the vegetative growth of strain VC. Later, purine-requiring auxotrophic mutants of M. xanthus, strain FBa were isolated by Hemphill and Zahler (52). This finding further confirmed the existence of a purine biosynthetic pathway in this organism. Detection of the individual enzymes involved in purine nucleotide de novo synthesis in this microbe have not yet been reported (up to this study).

The Early Steps of Purine Biosynthetic Pathway

The initial step in purine nucleotide synthesis de novo has been established to be the formation of phosphoribosylamine (PRA) from 5-phosphoribosyl-1-pyrophosphate (PP-ribose-P) and glutamine (36, 49) (reaction 1, Fig. 4). This step is catalyzed by the enzyme, PP-ribose-P amidotransferase (EC 2.4.2.14), and appears to be universal in all the biological systems that have been tested. PRA has never been isolated and characterized in any in vitro system because of its extremely unstability in aqueous solutions. Its synthesis was deduced from identification of the other reaction products, pyrophosphate and glutamine (80). The reaction is presumed to involve an isomeric inversion from the α-PP-ribose-P to the β-PRA. PP-ribose-P amido-transferase has been extensively purified from pigeon liver (17, 47, 49, 126), adenocarcinoma 755 (56) and certain microbes (98, 125). In the different in vitro systems that have been examined so far, PRA can

be formed not only through the mediation of the amidotransferase but also through an alternate first enzyme, ribose-5-phosphate amino-transferase (no EC number yet) which will be discussed later.

In the second de novo step, the carboxyl group of glycine reacts with the amino group of PRA, in the presence of phosphoribosyl-glycinamide (GAR) synthetase (EC 6.3.1.3) and maganesium ion, to form an amide linkage between glycine and the amino sugar. ATP is required as the energy source. The reaction products are GAR, ADP and phosphate (37, 38, 39, 107) (reaction 2, Fig. 4). The reverse reaction proceeds readily in vitro as is evidenced by the formation of equal quantities of glycine and ATP from GAR, ADP, Pi and the enzyme (11, 50). The enzyme involved in this reversible reaction has been purified from pigeon liver (50), Aerobacter aerogenes (99), and S. typhimurium (35) and its molecular weight is about 50,000 (35, 99). Nierlich and Magasanik (99) reported the absence of feed-back inhibition of the GAR synthetase of A. aerogenes at physiological concentrations of purines (99), however, a marked inhibition of this enzyme by ATP was found in S. typhimurium by Gandhi and Westby (35).

The third de novo step is the addition of a one-carbon formyl group to the free α-amino group of GAR and this step is catalyzed by formyl-GAR transferase (EC 2.1.2.2). The reaction product is formyl-GAR (38, 39) (reaction 3, Fig. 4).

The above mentioned steps are considered for the purposes of this study to be the early reactions of purine nucleotide de novo synthesis. Formyl-GAR is subsequently converted in most systems (by a series of

enzymatic reactions) to IMP, the first product of the pathway to possess a complete purine ring system.

An Alternative Synthesis of β-D-phosphoribosylamine (PRA)

In 1961, Nierlich and Magasanik (96) investigated PRA synthesis in A. aerogenes and E. coli and reported that PRA could be synthesized from ribose-5-phosphate, ATP, and ammonium ion when the appropriate enzyme and substrates were supplied (Fig. 5). They prepared a partially purified extract from A. aerogenes which used ribose-5-phosphate and ammonium ion but which would not convert ribose-5-phosphate to PP-ribose-P, the required substrate of PP-ribose-P amidotransferase. In 1965, however, the same authors (99) reported that this synthesis of PRA from ribose-5-phosphate, ATP, and ammonium ion was non-enzymatic in nature; ATP was not required.

Recently, an alternate first enzyme was discovered in other biological systems including wheat embryo (64), Ehrlich ascites tumor cells (55), and avian liver (113). Reem (113), for example, reported on the existence of two enzymatic routes in duck, chicken and pigeon liver, one involving L-glutamine and the other NH_4^+. She named the enzyme catalyzing the alternate route "ribose-5-phosphate amino-transferase," and purified it about 100-fold.

Another apparent enzymatic catalyzation of PRA from ribose-5-phosphate and ammonium ion was demonstrated by Le Gal et al. (75), using extracts of E. coli B. They found glutamine, ammonia, and carbamyl phosphate to be highly potent donors in the presence of

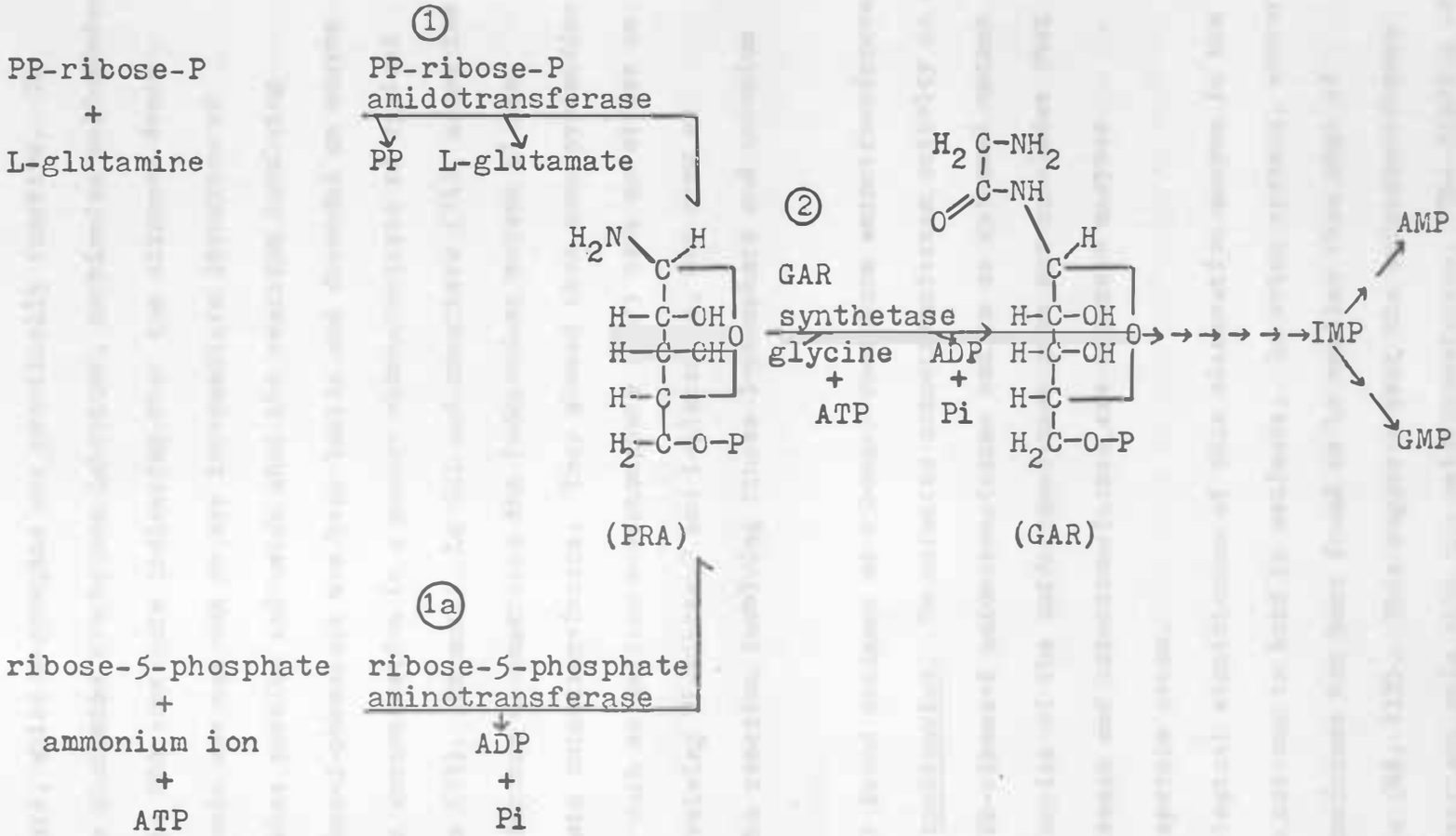


Fig. 5. Early reactions of de novo purine synthesis.

ribose-5-phosphate, while asparagine was practically inactive. In this system, the glutamine synthetase inhibitor, methionine sulfoximine, failed to inhibit PRA synthesis indicating that the nitrogen donor activity of ammonia was not due to any intermediate formation of glutamine. Further results indicated that the reaction involving ammonia and ribose-5-phosphate was heat labile and depended on enzyme and ammonium ion concentration in a manner characteristic of typical enzyme reactions (75). Recently, Le Gal and coworkers (74), using the same organism, further demonstrated the independent nature of the ribose-5-phosphate aminotransferase. They showed that some pyrimidine ribonucleotides such as uridine monophosphate (UMP) have no effect on the reaction involving PP-ribose-P and L-glutamine, but show an inhibition on the reaction involving ribose-5-phosphate and ammonium ion.

Conlin (19) found evidence of ribose-5-phosphate aminotransferase activity in S. typhimurium. He detected aminotransferase activity in a mutant lacking PP-ribose-P amidotransferase and in an episomal mutant carrying extra copies of the amidotransferase gene and concludes that the amidotransferase and aminotransferase are separate enzymes controlled by separate genes.

The physiological significance of this alternative enzyme in the above mentioned systems is hard to evaluate. In avian systems, activity of the aminotransferase has been found to be smaller than that of amidotransferase (99, 112). This suggests that the aminotransferase plays a less important role than the amidotransferase. The finding of

Conlin (19) that amidotransferase-less pur-F mutants still show an absolute requirement for purines although possessing the aminotransferase suggests that the alternate enzyme plays no major role in purine nucleotide de novo synthesis in vivo. To explain this discrepancy, Gots (40) has proposed that the intracellular ammonium or ribose-5-phosphate concentrations or both may be too low to meet the Km requirement of the aminotransferase or alternatively that both substrates may be high enough in concentration, but the aminotransferase may exist at critically low concentrations, resulting in a restriction of the potential use of the alternate route.

Side Pathways Branching from Purine Nucleotide De Novo Route

Thiamine biosynthesis

Much data has accumulated over a number of years that indicates a close relationship exists in microbial systems between purine metabolism and the biosynthesis of thiamine. Yura (165), for example, found that all mutants of S. typhimurium blocked before phosphoribosylaminoimidazole (AIR), the fifth intermediate of purine nucleotide de novo pathway (see Fig. 4), require both adenine and thiamine for growth and that mutants blocked after AIR require only adenine. It has also been shown that the wild type of the same organism is inhibited by high concentrations of adenine but shows a reversal of the inhibitory effect when thiamine or its pyrimidine moiety are provided (63). Such results have since been confirmed with mutants of S.

typhimurium (20, 94, 95) as well as with other microbes such as E. coli (88, 91), and A. aerogenes (88, 91). Other studies with the same organisms indicate that the biosynthesis of thiamine and its pyrimidine portion are actually inhibited by high concentrations of adenine (88). Newell and Tucker (95) demonstrated with S. typhimurium conclusively that adenosine inhibits the synthesis of the pyrimidine moiety of thiamine. They showed very clearly that the early steps of purine de novo synthesis coincide with the pathway of biosynthesis of the pyrimidine moiety of thiamine. AIR, the sixth de novo intermediate, was found to serve as a branch-point for both purine nucleotide and thiamine biosynthesis. Amino acids such as glutamine and glycine which participate in the formation of AIR are thus also involved in thiamine biosynthesis. Newell and Tucker (94) found that a methionine-deficient mutant of S. typhimurium synthesized very little pyrimidine moiety from AIR in the absence of methionine and this indicated that methionine was also required in the biosynthesis of thiamine. The pathway postulated by Newell and Tucker for the biosynthesis of the pyrimidine moiety of thiamine is outlined in Fig. 6.

Fig. 6 illustrates that a C_3 fragment is introduced into the molecule during the biosynthesis of the pyrimidine moiety of thiamine. The actual compound has not been identified as yet, but Newell and Tucker (95) have postulated it to be glycerol or α-glycerophosphate.

Since both purine nucleotides and the pyrimidine moiety of thiamine share common biosynthetic steps, it is safe to assume that those factors that regulate the early steps of purine nucleotide synthesis will also

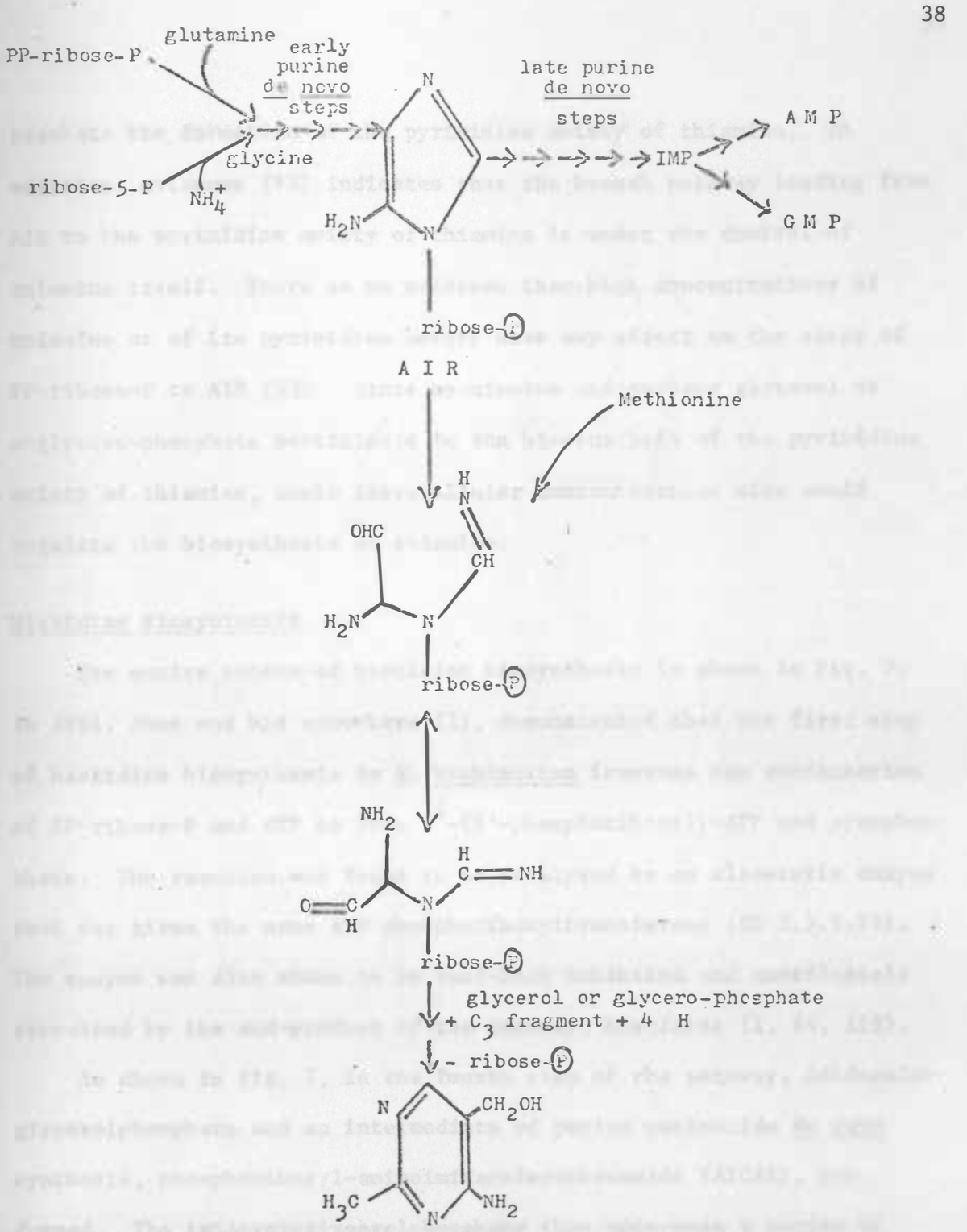


Fig. 6. Proposed pathway for the biosynthesis of the pyrimidine moiety of thiamine in bacteria (95).

regulate the formation of the pyrimidine moiety of thiamine. In addition, evidence (93) indicates that the branch pathway leading from AIR to the pyrimidine moiety of thiamine is under the control of thiamine itself. There is no evidence that high concentrations of thiamine or of its pyrimidine moiety have any effect on the steps of PP-ribose-P to AIR (53). Since methionine and perhaps glycerol or α-glycero-phosphate participate in the biosynethsis of the pyrimidine moiety of thiamine, their intracellular concentrations also would regulate the biosynthesis of thiamine.

Histidine biosynthesis

The entire scheme of histidine biosynthesis is shown in Fig. 7. In 1961, Ames and his coworkers (1), demonstrated that the first step of histidine biosynthesis in S. typhimurium involves the condensation of PP-ribose-P and ATP to form N^1-(5'-phosphoribosyl)-ATP and pyrophosphate. The reaction was found to be catalyzed by an allosteric enzyme that was given the name ATP phosphoribosyltransferase (EC 2.7.1.18). The enzyme was also shown to be feed-back inhibited and coordinately repressed by the end-product of the pathway, histidine (1, 84, 155).

As shown in Fig. 7, in the fourth step of the pathway, imidazole-glycerolphosphate and an intermediate of purine nucleotide de novo synthesis, phosphoribosyl-aminoimidazolecarboxamide (AICAR), are formed. The imidazoleglycerolphosphate then undergoes a series of subsequent enzymatic reactions to finally become histidine (86, 159), while the AICAR is salvaged and reused as a precursor of purine

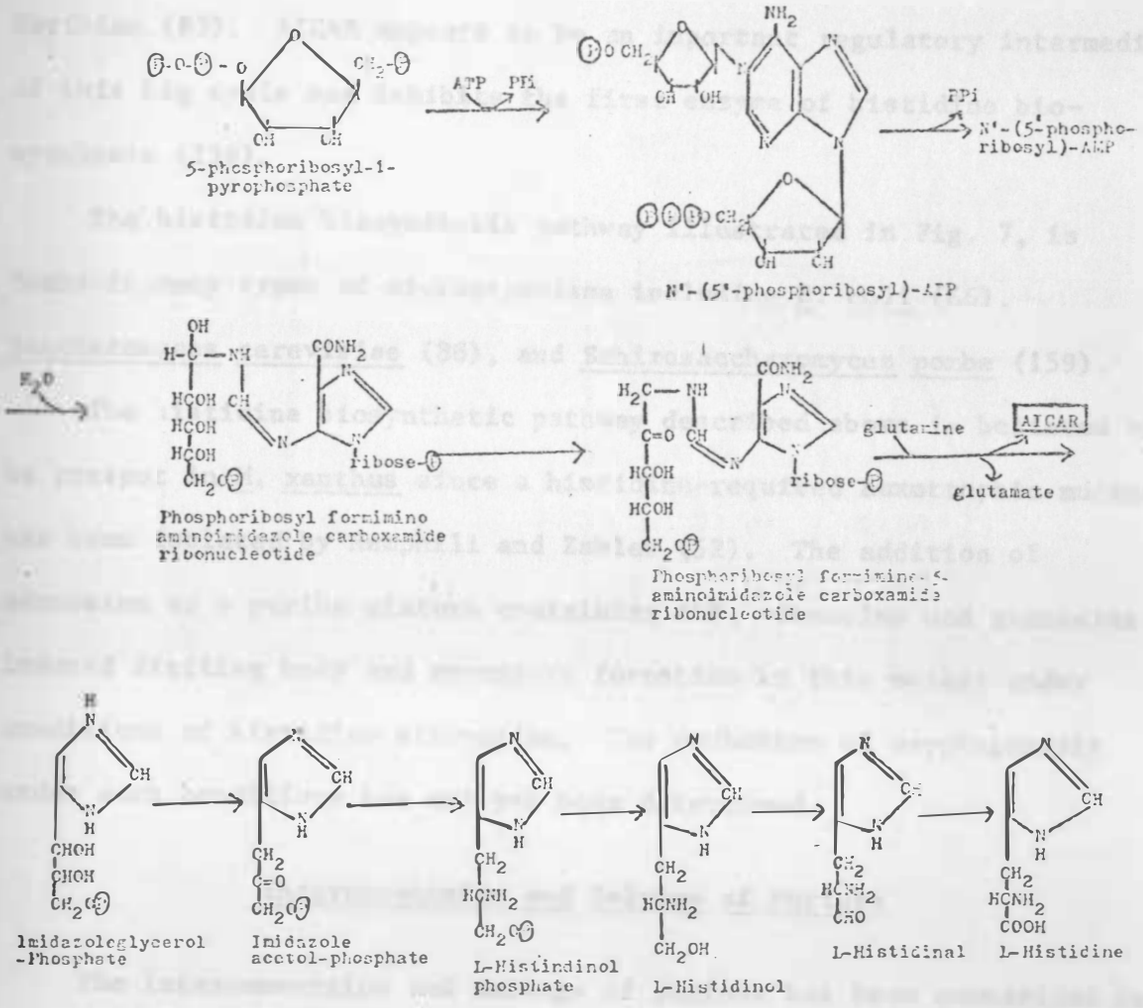


Fig. 7. The biosynthetic pathway of histidine. (P) denotes the phosphate group (53,76).

nucleotides (including ATP, the starting precursor of histidine biosynthesis). The histidine biosynthetic pathway viewed in this context comprises a branch of the "big purine cycle" of Magasanik and Karibian (83). AICAR appears to be an important regulatory intermediate of this big cycle and inhibits the first enzyme of histidine biosynthesis (159).

The histidine biosynthetic pathway illustrated in Fig. 7, is found in many types of microorganisms including E. coli (66), Saccharomyces cerevisiae (86), and Schizosaccharomyces pombe (159).

The histidine biosynthetic pathway described above is believed to be present in M. xanthus since a histidine-required auxotrophic mutant has been isolated by Hemphill and Zahler (52). The addition of adenosine or a purine mixture containing AMP, adenosine and guanosine induced fruiting body and myxospore formation in this mutant under conditions of histidine starvation. The mechanism of morphogenesis under such conditions has not yet been determined.

Interconversion and Salvage of Purines

The interconversion and salvage of purines has been summarized in recent reviews (6, 40, 90, 130). The pathways are shown in Fig. 8. IMP, the first complete purine nucleotide synthesized de novo, serves as a pivotal intermediate to form AMP along one branch and GMP along the other, each in a two-step, irreversible sequence. In the case of AMP synthesis, IMP is first converted to adenylosuccinate (S-AMP) by an aspartylation catalyzed by adenylosuccinate synthetase (EC 6.3.4.4)

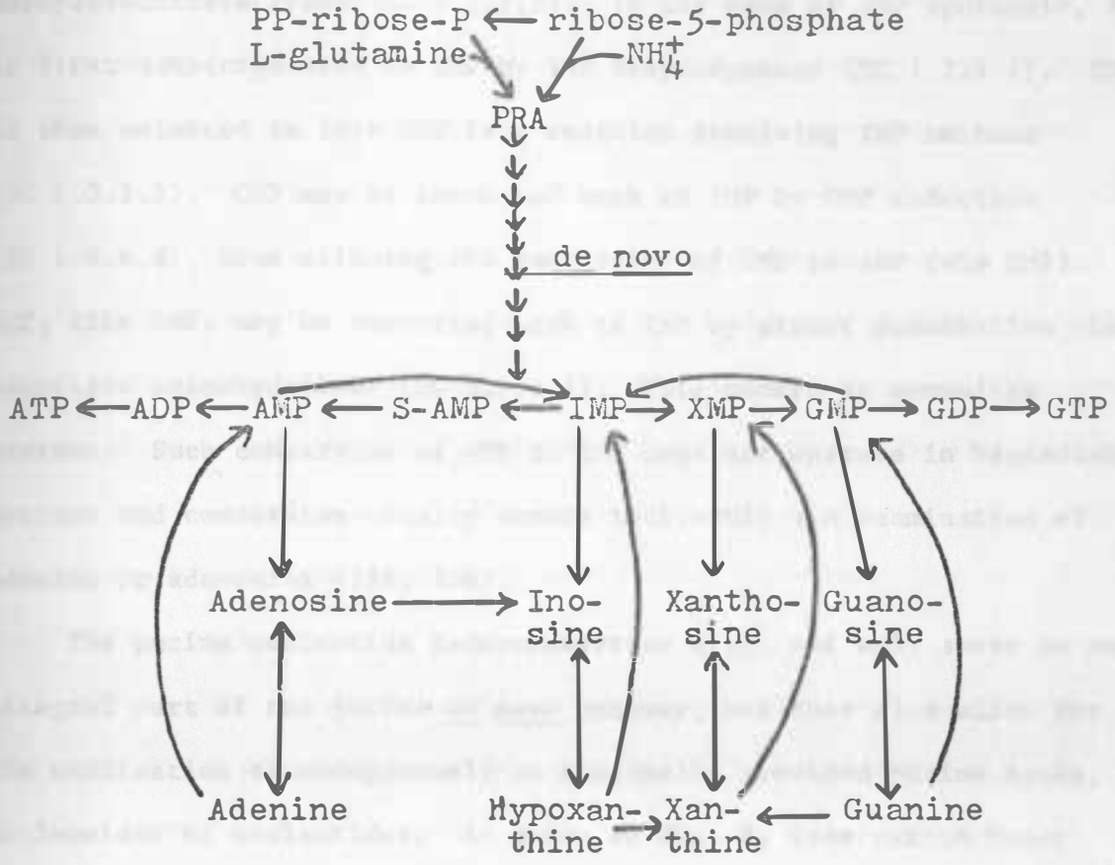


Fig. 8. Interconversion of purine nucleotides and salvage of aglycones and nucleosides (90).

and subsequently from S-AMP to AMP under the catalytic action of adenylosuccinate lyase (EC 4.3.2.2). In the case of GMP synthesis, IMP is first dehydrogenated to XMP by IMP dehydrogenase (EC 1.2.1.4). XMP is then aminated to form GMP in a reaction involving XMP aminase (EC 6.3.5.2). GMP may be converted back to IMP by GMP reductase (EC 1.6.6.8), thus allowing the conversion of GMP to AMP (via IMP). AMP, like GMP, may be converted back to IMP by direct deamination via adenylate aminohydrolase (EC 3.5.4.6). This occurs in mammalian systems. Such conversion of AMP to IMP does not operate in bacterial systems and conversion usually occurs indirectly via deamination of adenine or adenosine (116, 166).

The purine nucleotide interconversion steps not only serve as an integral part of the purine de novo pathway, but they also allow for the utilization of endogenously or externally provided purine bases, nucleosides or nucleotides. As shown in Fig. 8, free purine bases either formed endogenously from purine nucleotides or supplied exogenously are salvaged for reuse in nucleotide and nucleic acid biosynthesis. In bacterial systems such as B. subtilis (5) and Streptococcus faecalis (8), externally provided aglycones are converted to nucleotides in the presence of at least two enzymes, adenine phosphoribosyl-transferase (EC 2.4.2.7) and guanine-hypoxanthine phosphoribosyltransferase (EC 2.4.2.8). Xanthine can also be converted to its nucleotide, XMP, in the presence of a similar enzyme, hypoxanthine phosphoribosyltransferase (65). Purine nucleosides can also be converted to nucleotides, in this case by the phosphorylating action

of nucleoside kinases (15, 70). They may also be split by purine nucleoside phosphorylases (67, 68) to form inorganic phosphate and free purine bases. The aglycones, in turn, may be converted to nucleotides by the action of the appropriate purine phosphoribosyltransferases.

Berlin and Stadtman (5), using B. subtilis, discovered a special role for the purine nucleotide phosphoribosyl-transferases in purine uptake and incorporation. The uptake of a series of purines was found to be proportional to the activity of their nucleotide phosphoribosyl-transferases. The adenine phosphoribosyl-transferase has been found to be associated with a discrete locus, the membrane vesicles (5, 40). The uptake of adenine by membrane preparations is completely correlated with enzyme activity.

AMP or GMP, synthesized either de novo or by a salvage pathway, are usually further converted into ATP and GTP, respectively. This involves two successive transfers of high-energy phosphate groups from ATP to AMP or GMP, catalyzed by nucleoside monophosphate kinase and nucleosidediphosphate kinase, respectively (76).

M. xanthus was reported (138) to have the potential to lyse other host bacteria and to utilize them as a source of nutrients. Hemphill and Zahler (51) found that M. xanthus, FBa, possesses active nucleases that can hydrolyze both DNA and RNA and that the nuclease products can then be utilized as nutrients. Furthermore, purine-requiring auxotrophic mutants were found to be able to fulfill their nutritional requirements with purine bases, nucleosides, or nucleotides. This indirectly indicates the presence of the purine salvage pathway in this

organism. Dworkin (21), on the other hand, found that purines did not apparently stimulate the growth of M. xanthus and concluded that nucleic acids may not contribute significantly to the nutrition of this organism. So far, the presence of the purine salvage pathway in M. xanthus has not been directly demonstrated and its relationship, if any, with morphogenetic processes of the organism is still unknown.

Enzyme-Level Control (Feed-back Inhibition) of PRA Formation

An important biochemical regulatory mechanism is that of feed-back control in which an end product adjusts the rate of its own production by inhibiting the activity of the first enzyme unique to the biochemical pathway (151). The existence of feed-back inhibition in purine biosynthesis has been demonstrated in a number of different test systems, ranging from bacteria to mammals. The precise site of feed-back control of the purine de novo pathway has been pinpointed at the enzyme, PP-ribose-P amidotransferase, which catalyzes the first irreversible step of purine de novo synthesis. This enzyme-level control of PP-ribose-P amidotransferase has been demonstrated in various systems such as pigeon liver (17, 18, 126, 164), Ehrlich ascites cell (54), mouse tumor (adenocarcinoma 755) (56), human erythrocyte and rat liver (17, 164). In procarytic systems, feed-back inhibition of the PP-ribose-P amidotransferase of B. subtilis (125, 136), A. aerogenes (98), and S. typhimurium (40) has been observed. Nierlich and Magasanik (98) demonstrated that the A. aerogenes amidotransferase was

inhibited by AMP, ADP, GMP, GTP and IMP. Furthermore, they found that a mixture of AMP and GMP produced more inhibition than did either nucleotide alone, whereas inhibition by pairs of guanine nucleotides or of adenine nucleotides was additive. They concluded that the amidotransferase possesses two separate allosteric binding sites, one for 6-aminopurine nucleotides and one for 6-hydroxyl nucleotides and that there occurs an interaction between these sites leading to a synergistically enhanced inhibitory activity when two types of inhibitors are present simultaneously. In B. subtilis, the amidotransferase was found to be quite similar to that of A. aerogenes (136). The enzyme is strongly inhibited by AMP, ADP, GMP, and GTP; both adenine and guanine nucleotides are competitive against PP-ribose-P and both nucleotides induce a homotrophic substrate interaction for PP-ribose-P. Experiments in which both inhibitors were tested together suggests that there occurs separate sites for adenine and guanine nucleotides (136). The S. typhimurium amidotransferase is also similar to that of the A. aerogenes, it also is synergistically inhibited by the purine nucleotides. In addition, the S. typhimurium enzyme is also affected by AICAR, the eighth de novo intermediate, AICAR inhibits additively by itself and synergistically with either AMP or GMP (40).

The alternate first purine de novo enzyme, ribose-5-phosphate aminotransferase is also sensitive to feed-back inhibition by purines and their derivatives. Reem (113) discovered that purine nucleotides rather than purine bases are the true inhibitors of pigeon liver

ribose-5-phosphate aminotransferase. Inhibition of pigeon liver enzyme by AMP was shown to be noncompetitive with respect to ribose-5-phosphate. AMP and GMP in combination were found to cause no more than an additive effect indicating the absence of interacting binding sites. Le Gal et al. (75) have likewise demonstrated that both AMP and GMP feed-back inhibit the ribose-5-phosphate aminotransferase in E. coli. Tsai (148) demonstrated that ribose-5-phosphate aminotransferase of S. typhimurium is feed-back inhibited by AMP, ADP, GDP, and adenosine but not by ATP, GTP, GMP and free adenine.

Gene-Level Control (Repression and Derepression) of Purine Nucleotide De Novo Synthesis

Besides the enzyme-level control described in the preceding section, the purine de novo pathway is also subject to repression and derepression, a control that works at the level of gene expression rather than enzyme activity. Most of the studies on gene-level control have been done in microbiological systems. In A. aerogenes (97, 100) and S. typhimurium (157), for example, most of the enzymes responsible for purine de novo synthesis have been shown to be repressible and derepressible to varying degrees. Nierlich and Magasanik (97, 100) used wild-type A. aerogenes and various purine-requiring mutants grown with limiting or excess adenine or guanine and found that PP-ribose-P amidotransferase, GAR synthetase, formyl-GAR amidotransferase (reaction 4, Fig. 4), S-AMP synthetase (reaction 11, Fig. 4), IMP dehydrogenase (reaction 13, Fig. 4), and adenylosuccinate lyase (reaction 8 and 12,

Fig. 4) were under repression-derepression control. Of the enzymes, PP-ribose-P amidotransferase and formyl-GAR amidotransferase were found to be coordinately controlled (97, 100) while PP-ribose-P amidotransferase and GAR synthetase were found to be only semicoordinately controlled (98). Westby and Gots (157) carried out similar studies, with wild type and purine-requiring mutant of S. typhimurium that were grown on limiting or excess amounts of xanthine. They found both PP-ribose-P amidotransferase and GAR synthetase to be repressible-derepressible.

In B. subtilis, Nishikawa and coworkers (87, 101) found that IMP dehydrogenase was repressed by guanosine, S-AMP synthetase by adenosine; while IMP cyclohydrolase, PP-ribose-P amidotransferase and adenylosuccinate lyase were repressed by either adenosine or guanosine.

Levin and Magasanik (78) found that IMP dehydrogenase and IMP cyclohydrolase are both under repression-derepression control in S. typhimurium and A. aerogenes. Since guanine derivatives were much more effective in repressing IMP dehydrogenase than IMP cyclohydrolase, they suggested that separate repressor systems are involved in the control for the synthesis of these two enzymes.

MATERIALS AND METHODS

Chemicals

PP-ribose-P (sodium), D-ribose-5-phosphate (disodium), ATP (disodium), ADP (disodium), AMP (type V), adenosine, adenine, guanosine 5'-triphosphate (GTP; trisodium, type I), guanosine 5'-disphosphate (GDP; sodium, type I), GMP (sodium), guanosine IMP (type III), cytidine 5'-triphosphate (CTP; disodium, type IV), cytidine 5'-monophosphate (CMP; disodium), uridine 5'-triphosphate (UTP; sodium, type III), uridine 5'-monophosphate (UMP; disodium), 3':5' cyclic AMP, cocarbozylase (aneurine pyrophosphate: thiamine pyrophosphate chloride) and S-adenosyl-L-methionine iodine were obtained from Sigma Chemical Co. Guanine-HCl and thiamine-HCl were purchased from Nutritional Biochemicals Co. Acetone, magnesium sulfate, glycerol and toluene were from Fisher Scientific Co. Butyl alcohol (butanol), methyl alcohol anhydrous (methanol), and anhydrous ether were from Mallinckrodt Chemical works. Tris(hydroxymethyl)aminomethane-HCl and diethylamine were from Eastman Organic Chemical Co. Soli Mix I was from ICN Chemical and Radioisotope Division. Because of the considerable instability of PP-ribose-P, it was stored at -20 C in the powdered form and fresh solutions were prepared for each usage. Glycine-2-^{14}C, adenine-8-^{14}C and guanine-8-^{14}C were obtained from New England Nuclear Corp.

Bacterial Strains

M. xanthus FB, strain CW-1, was obtained from M. Dworkin, University of Minnesota. *M. xanthus* FBa, strain CW-2, was obtained from H. E. Hemphill, University of Washington and was originally derived from FB. *S. typhimurium*, strain LT-2 was kindly supplied by J. S. Gots, University of Pennsylvania.

Growth of Bacteria

In vitro enzyme studies

M. xanthus CW-2 and *S. typhimurium* LT-2 were employed in enzyme studies and for this purpose both were grown under identical conditions in the CT medium of Dworkin and Gibson (28). One liter screw-cap flasks (four vessels per *M. xanthus* CW-2 run) containing 500-ml quantities of CT medium were inoculated 1:50 with 24-h cultures of *M. xanthus* CW-2 or *S. typhimurium* LT-2, and the flasks were incubated at 30 C on a shaking water bath (gyrotory New Brunswick model G-76, speed setting of 4). *M. xanthus* CW-2 cultures (only vegetative log-phase cells) were incubated for 24 h, at which time their absorbancies were 0.37 to 0.59 at 520 nm (3.5×10^7 to 45×10^7 cells/ml). *S. typhimurium* LT-2 cultures were incubated for 13 to 18 h and gave a terminal absorbancy of 0.68.

In vivo purine-thiamine anabolism studies

M. xanthus CW-1 was not used in any of the enzyme studies but was employed in adenine, guanine and glycine cellular-uptake studies and in *in vivo* purine-thiamine synthesis tests.

CW-1 cells assumed to be fully repressed and feed-back inhibited for de novo purine synthesis were obtained by employing a special CT medium supplemented with either 100 µg/ml of adenine (to obtain cells repressed by adenine) or 100 µg/ml of guanine-HCl (to obtain cells repressed by guanine). The Bacto-casitone used to make this special medium (i.e. batch 504455) had been pre-tested (by M. Dworkin) so as to make certain that it would consistently support good (reproducible generation times of approximately 4.5 h) logarithmic growth. Certain batches of Bacto-casitone do not uniformly support such good growth and such batches were not used in these studies. The special CT medium (2% pre-tested casitone, other ingredients unchanged and supplemented with either adenine or guanine-HCl) was prepared in 100 ml quantities in 300 ml screw-cap, side arm (14 mm diameter) flasks and at the beginning of an experimental run the flasks were inoculated 1:14 from 20-24 h CT roller-tube cultures prepared from deep frozen stocks of vegetative cells or myxospores. The cultures were incubated at 30 C in a gyrotory shaking water bath (Metabolyte water bath shaker, New Brunswick Scientific Co., speed setting of 175 rpm) until the turbidity of the flask contents had reached an absorbancy of 0.4 at 520 nm (1.43×10^8 cells/ml). At this turbidity, these batch-culture cells are approximately at mid-log phase, have a generation time of 4.5-5 h and are termed in this report, fully repressed vegetative cells.

CW-1 cells assumed to be partially derepressed and only partly feed-back inhibited for de novo purine synthesis were obtained by using

a special dialyzed CT medium. Supplemental adenine and guanine were omitted from this medium and the pre-tested casitone that was employed was first dialyzed for 22 h against running tap water followed by 2 h against running deionized water to remove purine bases, nucleosides and nucleotides (other small molecules also removed). The final concentration of dialyzed casitone used in the medium (2%) was the same as that used above, however, the medium was sterilized by filtration (Nalge 0.45 μm disposable filter unit) rather than by autoclaving to prevent the formation of a precipitate that was found to result upon steam sterilization of dialyzed casitone (precipitate not formed on autoclaving of undialyzed CT medium). The dialyzed CT medium was prepared in 100 ml quantities in the same type of flask as mentioned above. At the beginning of an experimental run, flasks containing the medium were inoculated and incubated as described previously until the flask contents had attained a terminal absorbancy as before of 0.4 at 520 nm (1.1×10^8 mid-log phase cells/ml of 4.5-5.5 h generation time). The vegetative cells produced in this fashion are termed in this report, partially repressed vegetative cells.

Harvesting and Preparation of Vegetative Cells

In vitro enzyme studies

CW-2 vegetative cells to be extracted for PP-ribose-P amidotransferase and ribose-5-P aminotransferase were prepared in the following way. A 1- to 2-liter, 24-h culture (prepared as described above) was centrifuged (19,700 x g for 20 min at 0 C) and the sedimented

cells were washed twice with 30-ml quantities of sodium phosphate buffer (0.03 M, pH 7.5, 2mM 2-mercaptoethanol) prior to resuspension in 0.8 to 1.5 ml of the same buffer.

In vivo purine-thiamine anabolism studies

Fully and partially repressed CW-1 vegetative cells (prepared as described above in 100 ml batches) were harvested by centrifugation (5,900 x g for 15 min at 4 C) and were then either used directly as vegetative cells for uptake and synthesis studies or alternatively they were employed to produce fully or partially repressed myxospores (see below).

To prepare CW-1 vegetative cells for uptake or synthesis studies, the harvested cells (from 100 ml cultures) were first washed once with 160 ml of chilled potassium phosphate buffer (0.01 M, pH 7.2) containing 0.008 M $MgSO_4$ and then the washed cells were resuspended in 10 ml of the same phosphate-$MgSO_4$ buffer. No other supplemental ingredients were present in the buffer used to prepare partially repressed vegetative cells, however, to maintain the repression of fully repressed vegetative cells, 100 µg/ml of adenine (for cells repressed by adenine) or guanine-HCl (for cells repressed by guanine), respectively, were included in the buffer used to prepare such cells.

Myxospore Production

In vitro enzyme studies

A 1-liter culture of M. xanthus CW-2 vegetative cells, obtained as described above, was centrifuged at 19,700 x g for 20 min at 0 C

and the sedimented cells were washed once with 160 ml of a solution containing 1% casitone (Difco) and 0.008 M $MgSO_4$. The washed cells were suspended in 2 liters of fresh wash medium augmented with 0.5 M glycerol to induce myxospore formation (28). This suspension (in two, screw-cap, 1 liter flasks) was shaken at 30 C (gyrotory, New Brunswick model G 76, speed setting of 4) for 2.5 h to encourage complete sporulation. Microscope examination indicated that 90% or better of the cells had sporulated after 2.5 h. The myxospores were harvested by centrifugation (19,700 x g for 20 min at 0 C), washed once in 160 ml of 0.03 M sodium phosphate buffer at pH 7.5 (2 mM in 2-mercaptoethanol), and suspended in 0.7 to 2.0 ml of the same buffer for sonic treatment.

In vivo purine-thiamine anabolism studies

Adenine and guanine repressed CW-1 vegetative cells intended for myxospore conversion were glycerol-induced in a somewhat different way than that described above. Adenine repressed vegetative cells slated for myxospore production (harvested from 100 ml cultures as indicated above) were first washed once with 100 ml chilled 1% casitone (Difico batch 504455) containing 0.008 M $MgSO_4$ and 100 µg/ml of adenine. The cells were then suspended in 200 ml of myxospore induction medium which contains the same ingredients as the wash medium plus 0.5 M glycerol. Guanine repressed vegetative cells were washed and induced similarly except that adenine was replaced by gaunine-HCl in both the wash and induction media. To ensure adequate aeration for induction

in the adenine and guanine media (30), the suspended cells were vigorously shaken (Metabolyte water bath shaker, New Brunswick Scientific Co., 30 C, speed setting of 175 rpm) in a vessel with a large surface to volume ratio (1,200-ml Fleaker flask) and vigorously bubbled with air (MicroFerm Laboratory Fermenter air supply system, New Brunswick Scientific Co., air pressure set at 4.4). Different batches of vegetative cells were induced for various periods of time so as to produce fully repressed 1 h (immature), 2.5 h (morphologically mature), and 8 h (morphologically and physiologically mature) myxospores. The various lots of myxospores were harvested by centrifugation (5,900 x g for 15 min at 4 C).

Adenine repressed myxospores were selected for study of glycine-2-^{14}C uptake. To prepare such cells for this type of study, myxospores, glycerol induced in the presence of adenine (harvested as described above), were washed once with 160 ml chilled potassium phosphate buffer (0.01 M, pH 7.2) augmented with 0.5 M glycerol, 0.008 M $MgSO_4$ and adenine (100 µg/ml) and the cells were subsequently suspended in 10 ml of the same solution.

Adenine repressed myxospores were also used to study adenine-8-^{14}C uptake. These myxospores were prepared in a similar manner to that described above except that unlabelled adenine was omitted from the final 10 ml of suspending buffer (adenine was present, however, in wash).

Guanine repressed myxospores were used to study guanine-8-^{14}C uptake. The myxospores (glycerol induced in presence of guanine-HCl)

were prepared as above except that adenine was replaced by gaunine-HCl (100 µg/ml) in the wash medium and guanine was omitted (in unlabelled form) along with adenine in the final 10 ml of suspending buffer.

All of the myxospore batches were sonicated once for 20 sec (Branson ultrasonifier, Model 9110, using small probe with 3 mm diameter tip, power setting of 3) to break up sticky clumps of spores and produce a uniform suspension. Individual myxospores are resistant to such treatment and do not rupture (142). Each lot of myxospores was checked under the phase microscope for cellular uniformity before being used in glycine, adenine or guanine uptake studies.

Partially repressed myxospores were obtained from partially repressed CW-1 vegetative cells in a manner similar to that described for obtaining fully repressed myxospores. Added adenine and guanine, however, were omitted from the pre and post myxospore wash, induction and suspending media (to maintain partial repression). Casitone that was utilized in certain of these solutions was 2% dialyzed (filter sterilized). Each lot of partially repressed myxospores was also treated with sonic oscillation for 20 sec to break up sticky clumps of spores and thereafter examined for uniformity under the phase microscope. Partially repressed myxospores so obtained were used either for uptake studies or for <u>in vivo</u> purine-thiamine synthesis tests.

Myxospore Germination

In vivo purine-thiamine anabolism studies

Certain fully and partially repressed 8 h myxospore batches were used to obtain germinating 8 h myxospores. After glycerol induction for 8 h as previously described (with or without adenine, guanine or dialyzed casitone depending upon cell type), the myxospore batches destined for germination were washed and resuspended exactly as described previously for fully (adenine or guanine-HCl) and partially (dialyzed casitone but no purines) repressed myxospores except that glycerol was omitted from the wash and suspending media. Under these conditions, myxospores will germinate (110).

Preparation of Vegetative Cell Extracts for Amido- and Aminotransferase Assay

In vitro enzyme studies

The thick paste of washed CW-2 vegetative cells (obtained as described above) was either sonically treated immediately or frozen overnight (-20 C) and sonically treated the following day. Sonic treatment was accomplished with a Bronswill Biosonic III sonerator, by employing the small (red-banded) probe at a setting of 35 intensity units. A cell-free extract that had combined PP-ribose-P amidotransferase, ribose-5-P aminotransferase, and GAR synthetase activities was obtained when the sonic treatment consisted of 3 to 10-s bursts interrupted by 60-s cooling breaks in an ice bath. Samples of not more than 1 to 2 ml were sonically treated at a time.

After sonic treatment, the crude extract was clarified by centrifugation at 48,000 x g for 30 to 50 min at 0 C. Particulate material was discarded, whereas the amber supernatant fluid, which contained the enzymes, was saved and stored at -20 C.

S. typhimurium extracts were prepared in an identical way.

Preparation of Myxospore Extracts for Amido- and Aminotransferase Assay

In vitro enzyme studies

Cell-free myxospore extracts of M. xanthus CW-2 were prepared in a similar manner, by employing a thick myxospore slurry as starting material (obtained as described above). More extensive sonic treatment was required, however, to break open the thick-walled myxospores. Thus, each myxospore suspension was exposed to 10 to 30-s bursts as above and then, after resuspension with small glass beads (1 part 0.1- to 0.11-mm beads to 4 parts myxospore suspension), to 5 additional 30-s bursts (under the same conditions). Particulate-free extract was prepared by centrifugation as above.

The protein content of all extracts was determined by the method of Lowry et al. (79).

PP-ribose-P Amidotransferase-dependent GAR Formation (Assay 1)

In vitro enzyme studies

An assay was devised after the method of Westby and Gots (157) and Gandhi and Westby (35) to measure the conversion of PP-ribose-P and

L-glutamine to GAR (reaction 1 and 2, Fig. 5). Test and control incubation mixtures contained 50 μ mole of tris (hydroxymethyl) aminomethane-hydrochloride buffer (pH 8.0), 5 μ mole of PP-ribose-P (sodium), 10 μ mole of L-glutamine, 1 μ mole of ATP, 1.2 μ mole of glycine-2-^{14}C (0.125 μ Ci/μ mole), 5.5 μ mole of $MgCl_2$, and crude extract (3.0 mg of protein vegetative extract or 2.8 mg of protein myxospore extract) in a final volume of 0.4 ml. Test mixtures were incubated at 30 C for 10 min, whereupon the coupled reaction was stopped and the protein was denatured by the addition of 0.05 ml of 50% trichloroacetic acid. Control mixtures received trichloroacetic acid at zero time of incubation (prepared in an ice bath) and were centrifuged with the test mixtures to remove the denatured proteins. After centrifugation, 0.2-ml samples were removed from the supernatant fractions and diluted with 1.5-ml quantities of 0.01 N HCl, and the totals were added to Dowex 50 ammonium (8% cross-linkage, 200 to 400 mesh) columns (0.5 by 7 cm). GAR (and formyl-GAR) eluted from such columns with 3.0 ml of 0.01 N HCl, whereas unutilized glycine remained attached to the column resin under these conditions. Samples from the column elutions (4.7 ml per column) were spotted on planchets, the liquids were removed by evaporation under an infrared lamp, and the radioactivity on the planchets was determined with a Nuclear-Chicago end-window gas flow counter. By subtracting control counts and applying a factor derived from the specific activity of the glycine-2-^{14}C used, the nanomle of GAR produced in a test mixture were ascertained.

Preliminary studies indicated that 3.0 mg of protein (or less) of crude vegetative extract of M. xanthus CW-2 and 10 min or less of incubation at 30 C were on scale for linear GAR production.

Ribose-5-phosphate Aminotransferase-dependent GAR Formation (Assay 1a)

In vitro enzyme studies

An assay similar to the above was devised to measure the coupled ribose-5-phosphate aminotransferase and GAR synthetase conversion of ribose-5-phosphate and NH_4Cl (reactions Ia and 2, Fig. 5). The assay mixture was identical to the above-described system except that the PP-ribose-P and L-glutamine were replaced by ribose-5-phosphate (disodium) and NH_4Cl at corresponding concentrations. Test mixtures were incubated for 30 min at 30 C. Subsequent to incubation, the methods were identical to the above procedures, and zero-time controls were run.

Preliminary tests suggested that 3.0 mg of protein (or less) of crude extract and 30 min or less of incubation at 30 C were on scale for linear GAR production.

Measurement of Glycine Uptake in Strain CW-1

In vivo purine-thiamine anabolism studies

A modification of the method of White (personal communication), and Ramsey and Dworkin (110) was employed to measure the rate of uptake of glycine by vegetative cells and myxospores of CW-1. Fully and partially repressed vegetative cells and myxospores (1 h, 2.5 h, 8 h

and germinating 8 h), prepared as previously described, were all identically handled in the uptake studies. In each case, duplicate 1.5 ml samples (from 10 ml lot) of each cell type (or in some cases duplicates from replicate batches of same cell type) were added (samples run in sequence not simultaneously) to 12.9 ml quantities of the same solution used for the final suspension of that particular cell type (in a 200 ml Nalge centrifuge bottle). Each cell-suspension sample was pre-incubated for 5 min in a 30 C shaking water bath (Metabolyte water bath shaker, New Brunswick Scientific Co., speed setting of 175 rpm). After the 5 min preincubation, 0.6 ml of glycine-2-^{14}C (specific activity of 5.97 mCi/mmole) was added to each system to give a final concentration of glycine-2-^{14}C of 1 µCi/ml. At various intervals 1.0 ml samples were taken and the suspended cells were checked for radioactivity. This was accomplished through a membrane pad filtering technique. Thus at 0, 5, 10, 15, 30, 45, 60, 90, 120 min after the addition of radioactive glycine, 1.0 ml samples were rapidly filtered through 0.22 µm pore diameter membrane pads (Millipore catalog number GSWP02500, 25 mm diameter). A 5 or 10 ml syringe connected to a Swinney adaptor holding the filter pad was employed to obtain rapid filtration of the small volume samples. In this filtration system, vegetative cells and myxospores are retained on the surface of the membrane while liquid and soluble components pass through the bacterial-proof pores of the pad. Following individualized filtration of the timed 1.0 ml cell-suspension samples, the cell-laden filter pads were washed (using Swinney system as above)

with 3 ml of the same buffer used for the final suspension and the wet pads were dried in a 60 C oven overnight or at room temperature for 4-7 days. Cell sample filtrates were discarded. The optical density (O.D.) of each cell suspension was also measured during glycine uptake (Spectronic 20, 520 nm) to monitor O.D. changes.

Each dried filter pad containing radioactive cells was placed in a glass scintillation vial (Packard catalog number 60010075, 20-ml volume) containing 10 ml of scintillation fluid (5 g Soli Mix I per 1 liter toluene). The level of radioactivity (cpm) on each pad was determined by using a Packard Tri-Carb liquid scintillation counter (Model 3320).

Measurement of Adenine and Guanine Uptake in Strain CW-1

In vivo purine-thiamine anabolism studies

Fully and partially repressed CW-1 vegetative cells and myxospores (1 h, 2.5 h, 8 h and germinating 8 h), prepared as described above, were examined for exogenous adenine and guanine uptake. The same membrane filtering procedure as used above to measure glycine uptake was also employed to measure uptake of adenine and guanine. In the case of adenine uptake, however, glycine-2-^{14}C was replaced by adenine-8-^{14}C (specific activity of 53.5 mCi/mmoles) in the incubation system and in the case of guanine uptake, glycine-2-^{14}C was replaced by guanine-8-^{14}C (specific activity of 52.01 mCi/mmoles).

Data Processing of Scintillation and Cell Count Results

In vivo purine-thiamine anabolism studies

A special computer program (in BASIC) was devised (Lanny Nusz) to expedite the accurate and rapid conversion of cpm's of glycine-2-^{14}C, adenine-8-^{14}C or guanine-8-^{14}C taken up/X number of cells/time of incubation. The program was designed for use on the model 9830 Hewlett-Packard minicomputer (8 K read/write words, extended input/output ROM) and was electronically stored in the 8 K memory bank of the instrument. Important input data also entered into the memory bank and necessary for the proper working of the program included scintillation counter cpm's, numbers of viable CW-1 cells/ml of culture, min of incubation, quench curve information and isotope specific activity. Output data from the computer was programmed to be typed out in graph form (as p moles of substance taken up/10^9 cells versus time of incubation) by a teletype interfaced to the computer. A cell count value of 10^9 was chosen because the results of a recent study similar to this one (45) and referred to in the "DISCUSSION" are also reported in these terms.

Measurement of De Novo Purine-Thiamine Synthesis in Strain CW-1: Thin Layer Chromatography

In vivo purine-thiamine anabolism studies

Partially repressed vegetative cells and myxospores (1 h, 2.5 h, 8 h and germinating 8 h), prepared as described above, were given

glycine-2-^{14}C for a short period of time to allow for the *in vivo* uptake and incorporation of this purine nucleotide precursor (as part of purine ring) into trichloroacetic acid soluble purine nucleotides and thiamine. Each 10 ml of cell suspension (1.1×10^9 cells/ml) was given 0.045 mCi of glycine-2-^{14}C (specific activity of 5.97 mCi/mmole) and subsequently incubated at 30 C in a shaking water bath (as above) for 90 min (50 min for germinating 8 h myxospores) to allow for glycine uptake and usage in purine *de novo* synthesis. After 90 min (50 min for germinating 8 h myxospores), each cell suspension was harvested by centrifugation (5,900 x g) for 15 min at 4 C and washed once with 160 ml of the same buffer used for final suspension. The sedimented cells were then treated with 3 ml of cold 10% trichloroacetic acid in 30% methanol in an ice bath (with occasional mixing) to liberate trichloroacetic acid soluble components from the cells. After 50 min of such treatment, a precipitate that formed was removed by centrifugation (27,000 x g for 30 min at 0 C) and was discarded (cell walls and trichloroacetic acid precipitable nucleic acids and proteins). The supernatant was extracted five times with 9 ml cold water-saturated diethylether to remove the trichloroacetic acid (9, 45). Various samples so obtained were frozen overnight at -70 C and were lyophilyzed on the following day (VirTis). The lyophilyzed residues were diluted with 0.15 to 0.2 ml distilled water and were either used directly for thin layer chromatography or were frozen (-70 C) and used later.

Measurement of De Novo Purine-Thiamine Synthesis in Strain CW-1: Radioautography

In vivo purine-thiamine anabolism studies

Aliquots (10-30 µl) of the lyophilyzed, trichloroacetic acid-soluble extracts of CW-1 prepared as described above were applied to untreated thin layer chromatoplates (20 x 20 cm plastic backed, Mackerey-Nagel polygram Cel 300, distributed by Brinkmann Instrument Inc.) for one or two dimensional chromatography. Control chromatoplates were spotted with extracts augmented with authentic AMP, ATP, GMP, GTP, CMP, UTP, thiamine-HCl, thiamine pyrophosphate, or S-adenosyl-methionine (10 µl of extract mixed with 10 µl of given substance and mixture spotted). Each sample was spotted on a separate chromatoplate (about 30 mm from bottom edge) in a stepwise fashion with intermittent drying between applications with cold air from a hair dryer. After the spots had completely dried, the thin layer plates were first chromatographed (in the ascending fashion, glass tank walls lined with absorbent paper saturated with solvent) with solvent I (100 ml):n-butanol:acetone:diethylamine:water (10:10:2:5). This solvent system moves amino acids, including glycine, away from the origin but does not move or only slightly moves nucleotides (112). As soon as the solvent front had advanced the desired distance (14-16 cm) up a given thin layer plate, the chromatogram was removed from the tank and dried at room temperature.

After the thin layer plates had dried, they were checked under ultraviolet-light (Mineralight, UVS-11, Ultra-violet Products, Inc.,

San Gabriel, California) for UV light-absorbing and reflecting spots and their UV coloration (i.e. purple, blue, blue-green). The spots as well as the solvent front and origin were lightly marked with a No. 2 pencil. Certain of the one-dimensional chromatograms were checked for radioactive spots by radioautography. This was accomplished by placing the chromatogram of interest face down in intimate contact with an unexposed sheet of face up X-ray film (Kodak single-coated medical X-ray film, blue sensitive code 5B54) (weight placed on top of combination) and exposing the film to the chromatogram in the dark for 7 days. Exposed films were developed according to standard procedure. Once developed, films could be overlaid on their respective chromatograms so as to superimpose radioactive and UV spots.

Following one dimensional thin layer chromatography (and when necessary the radioautography) the top part of each chromatogram just below the pH front was cut off and discarded. Each top-sheared chromatoplate was then chromatographed in the second dimension against 1.5 M potassium phosphate, pH 3.4 (100 ml). Conditions were similar to those indicated above. The second solvent system is known to separate nucleotides according to the number of their charges (charges essentially equivalent to the number of phosphate groups attached to the nucleoside) (120). Following two dimensional chromatography, the chromatograms were again examined for UV and radioactive spots by the same procedures used above.

Measurement of HPN and MS Synthesis in Strain CW-1

In vivo purine-thiamine anabolism studies

To check for the presence or absence of HPN and MS in partially repressed vegetative cells and myxospores of CW-1 (1 h, 2.5 h, 8 h and germinating 8 h) extracts were prepared from such cells (by method described above) and analyzed by one dimensional thin layer chromatography. The solvent system used for this purpose was 1.5 M potassium phosphate, pH 3.4 (120). The procedures for thin layer chromatography and radioautography were the same as used above.

RESULTS

PP-ribose-P Amidotransferase and Ribose-5-phosphate Aminotransferase of Vegetative Cells of M. xanthus CW-2

A PP-ribose-P amidotransferase route and an independent ribose-5-phosphate aminotransferase route to PRA and GAR have been demonstrated in the de novo purine synthesis of a number of higher and lower organisms (40, 90, 130, 163). The first such indication that this also holds true for M. xanthus comes from my findings (Table 3). Crude M. xanthus CW-2 vegetative extract was tested in the presence and absence of various de novo substrates for its ability to produce GAR via the amido- and aminotransferase routes.

When exogenous PP-ribose-P was missing from an amidotransferase test mixture (assay 1), little or no GAR was produced (Table 3, c). On the other hand, when exogenous L-glutamine was missing from such a mixture, a small but significant amount of GAR was produced (Table 3, b). This can be attributed to small amounts of endogenous L-glutamine being present in the crude extract, possibly as a result of protein breakdown by the sonic treatment.

When either ribose-5-phosphate or NH_4Cl was missing from an aminotransferase test mixture (assay 1a), no GAR was produced (Table 3, f and g).

When the nitrogen donors (L-glutamine and NH_4Cl) were cross-tested at comparable concentrations with the two ribosyl substrates, positive

Table 3. Effect of various substrates on the PP-ribose-P amidotransferase and ribose-5-phosphate aminotransferase-dependent GAR capacity of crude vegetative extracts of *M. xanthus* CW-2.

Additions[a]	nmole of GAR per mg of protein per min
a. PP-ribose-P, L-glutamine	0.61
b. PP-ribose-P	0.13
c. L-glutamine	0.037
d. PP-ribose-P, NH_4Cl	0.62
e. Ribose-5-phosphate, NH_4Cl	0.18
f. Ribose-5-phosphate	0
g. NH_4Cl	0
h. Ribose-5-phosphate, L-glutamine	0.05

[a]To incomplete assay 1 (a-d) or 1a (e-h) reaction mixture were added one or more of the indicated substrates at their usual concentration. The assay values represent the averages of two separate runs.

responses were obtained in both cases. NH_4Cl was just as good as L-glutamine as a nitrogen donor for the amidotransferase (Table 3, d), but L-glutamine was a much less effective nitrogen donor than NH_4Cl for the aminotransferase (Table 3, h). The different patterns of response to the two nitrogen donors in the two types of assay mixtures suggest that two PRA-forming enzymes (i.e., PP-ribose-P amidotransferase and ribose-5-phosphate aminotransferase) are present in M. xanthus CW-2.

Effect of Freezing on the Amido- and Aminotransferase

Another indication that two PRA-forming enzymes exist in M. xanthus CW-2 comes from the differential effect of -20 C storage on the amido- and aminotransferase activities of cell extracts.

One sample of crude extract, after 42 days of storage at -20 C, had entirely lost (100% decline) its catalytic capacity to produce GAR via the amidotransferase route, but had only partially lost (49% decline) its capacity via the aminotransferase route. Two other batches of crude extract both lost 10% of their amidotransferase-dependent, GAR-forming capacity after 2 days of storage at -20 C; however, a third batch held at this temperature lost none of its aminotransferase-dependent capacity even after 29 days of storage.

Comparison of M. xanthus CW-2 and S. typhimurium LT-2

To determine how M. xanthus CW-2 compared with other bacteria in purine de novo synthesis at 30 C, S. typhimurium LT-2 was chosen as a

bacterial prototype, and it and FBa were grown, harvested, extracted, and tested for their amido- and aminotransferase-dependent GAR capacities in an identical fashion. Under these circumstances, LT-2 extract catalyzed the production of 0.60 nmol of GAR per mg of protein per min via the amidotransferase (assay 1) and 0.109 nmol of GAR per mg of protein per min via the aminotransferase (assay 1a). Correspondingly, the CW-2 extract catalyzed the production of 0.69 nmol of GAR per mg of protein per min via the amidotransferase and 0.19 nmol of GAR per mg of protein per min via the aminotransferase. These results suggest (assuming there were saturating levels of GAR synthetase in all assayed extracts) (99, 113, 114) that both enzymes are present at nearly identical levels in both organisms.

Effect of End-products on the Amido- and Aminotransferase

The amido- and aminotransferases of various organisms are subject to feedback inhibition by various end-products of the purine nucleotide *de novo* pathway (40, 57, 75, 113, 114, 147, 162, 163). Table 4 illustrates the *in vitro* effects of various purine nucleosides and nucleotides both on the amido- and on the aminotransferase-dependent GAR capacity of CW-2 (assays 1 and 1a). The effect of pyrimidine nucleotides is also indicated.

In designing the end-product tests, the GAR synthetase activity of CW-2 extracts (coupling enzyme in amido- and aminotransferase assays) was assumed not to be inhibited by any of the end-products at the concentration tested or was inhibited to only a small extent (99,

Table 4. Inhibitory effects of various purine and pyrimidine compounds on the PP-ribose-P amidotransferase and ribose-5-phosphate aminotransferase activities of M. xanthus CW-2 vegetative extracts.

Inhibitor	Inhibition[a] (%)		
	Amidotransferase	Aminotransferase	
	(6.25 mM inhibitor)	12.5 mM inhibitor	25 mM inhibitor
Adenosine	75.9		74.1
AMP	94.1	88.6	100.0
ADP	95.8		57.4
ATP	1.9		28.8
3':5' cyclic AMP	33.6 (30.6)[b]	57.6 (56.7)[b]	
Guanosine	48.0		2.2
GMP	100.0	16.5	48.4
GDP	98.1		83.6
GTP	60.5		58.7
IMP	69.8		29.1
UMP	19.6		43.2
CMP	12.7		15.7
CTP	0.9		100.0[c]

[a]Most of the inhibitors were tested at least twice, and the values cited represent averages.

[b]Values in parentheses give cyclic AMP inhibitions of corresponding myxospore enzymes under comparable conditions.

[c]Assay with CTP gave a reading considerably below the base-level, zero-time control value.

130). On the basis of this assumption, some distinctive properties of both the amido- and the aminotransferases became evident from the results. AMP, ADP, GMP, GDP, at the concentration tested (6.25 mM), were equally effective inhibitors of PP-ribose-P amidotransferase. Adenosine, guanosine, IMP, and GTP, on the other hand, were less effective inhibitors, whereas ATP was a noninhibitor at the same concentration. The pyrimidine nucleotides (UMP, CMP, CTP) exerted only a slight inhibitory effect.

The ribose-5-phosphate aminotransferase of CW-2 displayed a somewhat different pattern of nucleoside and nucleotide inhibition than did the amidotransferase (Table 4, Fig. 10). First, higher concentrations (12 to 25 mM) of the end-products were required to elicit comparable inhibitory effects of enzyme activity under the assay conditions (12.5 mM ribose-5-phosphate and 25 mM NH_4Cl). Second, only AMP and cyclic AMP, of all the end products, were quantitatively effective inhibitors (at the concentrations tested) of the enzyme. GDP, adenosine, ADP, GMP, GTP, and IMP were less potent inhibitors of the aminotransferase, whereas guanosine and ATP were completely without effect. Of the pyrimidine nucleotides, only UMP seemed to have a significant inhibitory effect. CTP produced an apparent 100% inhibition, but this is considered to be artifactual because the assay with CTP gave a reading considerably below the base-level, zero-time control value.

The inhibitory effects of various concentrations of AMP and GMP on both the amido- and the aminotransferase are indicated in Fig. 9 and 10. The closed circle curves in each of these figures depict inhibition

Fig. 9. Dosage effect of AMP and GMP on the PP-ribose-P amidotransferase activity of vegetative and myxospore extracts of M. xanthus CW-2. Various quantities of AMP and GMP were singly added to a series of assay 1 reaction mixtures as described in MATERIALS AND METHODS, the amount of GAR produced in each case was determined as described, and the inhibitions were calculated. Each individual concentration of AMP and GMP was separately tested three to four times, and the curves represent a composite of the results. Symbols: ● , vegetative extract; □ , myxospore extract.

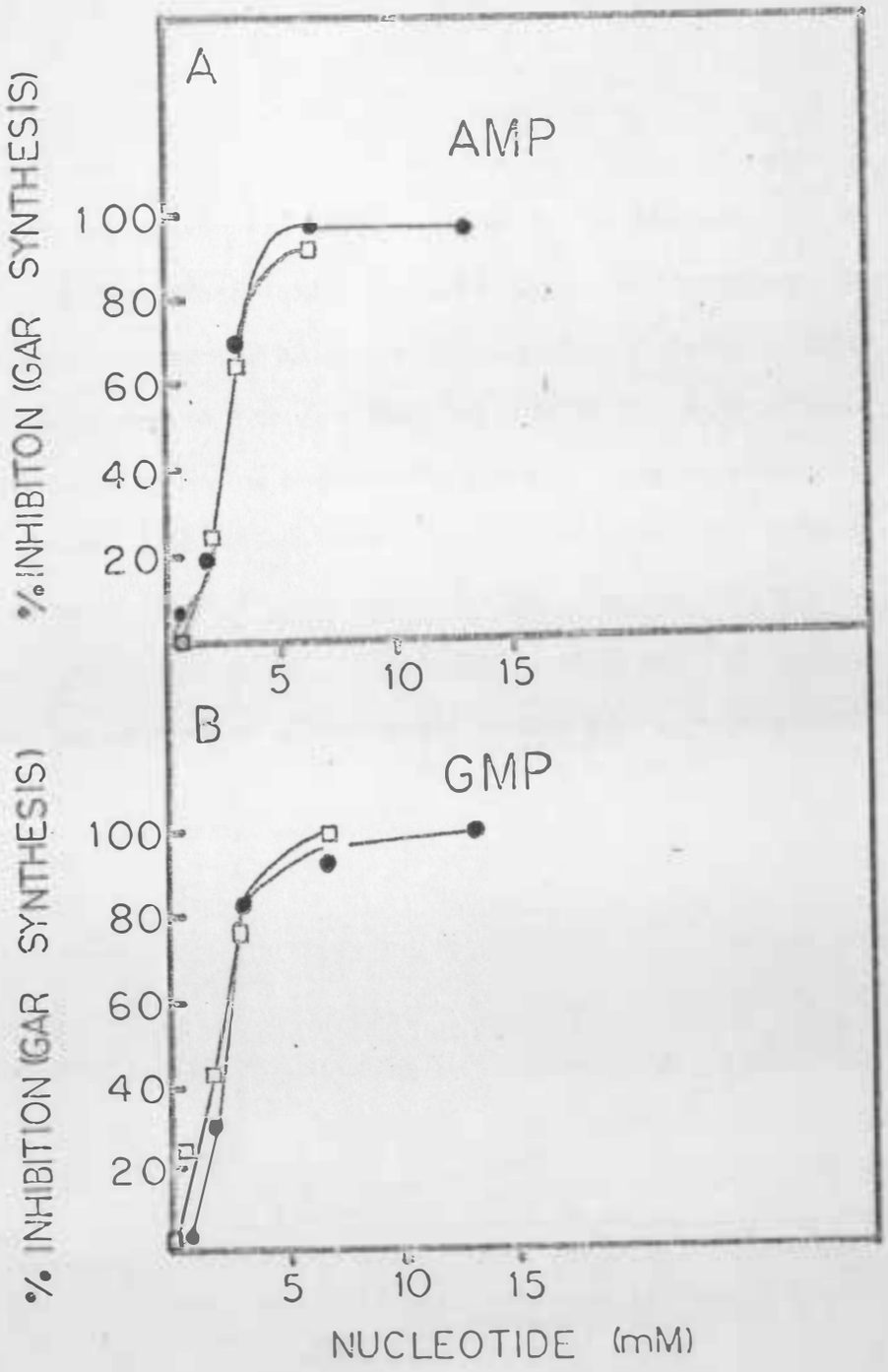
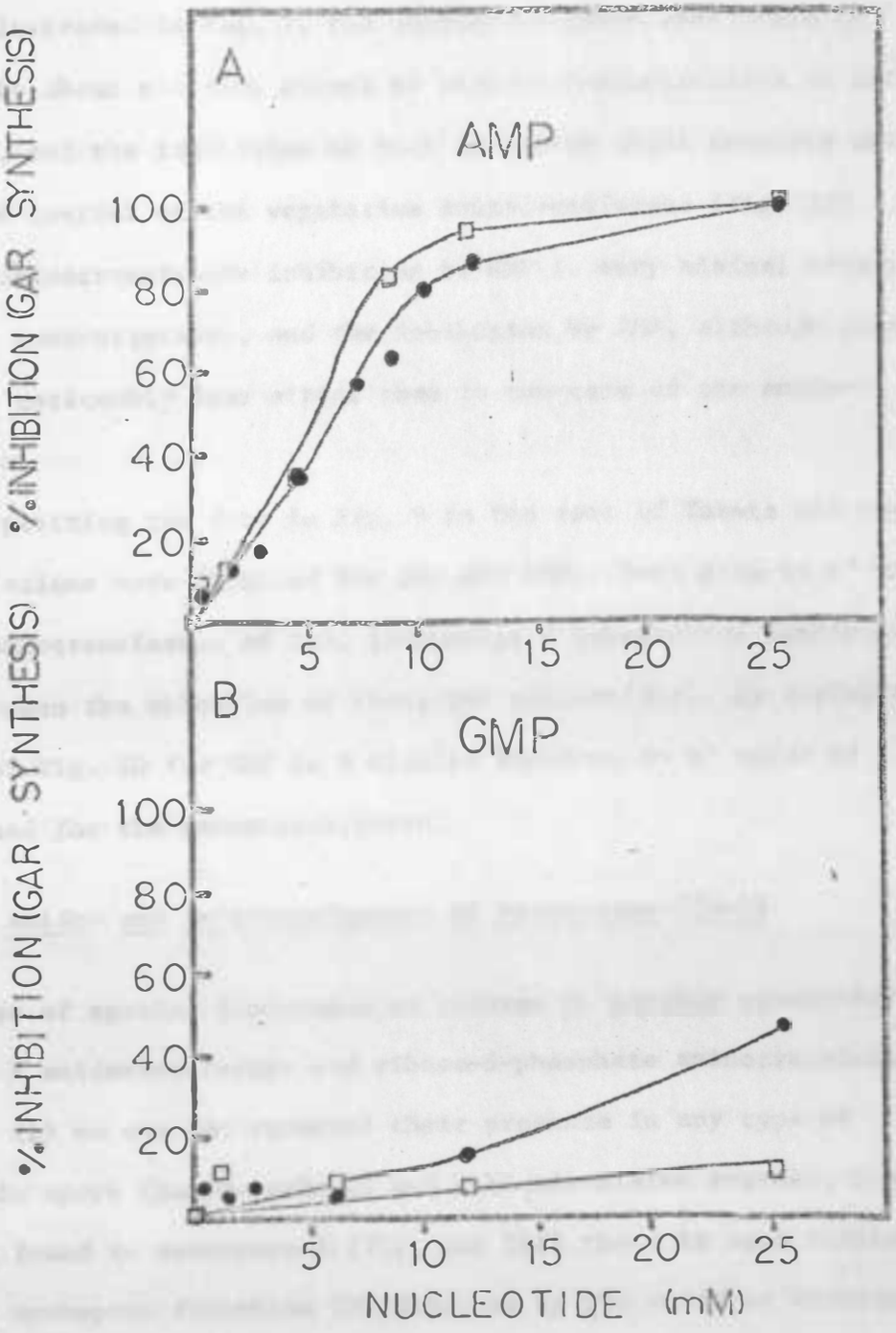

A
AMP
100
80
60
40
20
5
10
15
% INHIBITON (GAR SYNTHESIS)
B
GMP
100
80
60
40
20
5
10
15
% INHIBITION (GAR SYNTHESIS)
NUCLEOTIDE (mM)

Fig. 10. Dosage effect of AMP and GMP on the ribose-5-phosphate amino-transferase activity of vegetative and myxospore extracts of M. xanthus CW-2. Various quantities of AMP and GMP were singly added to a series of assay la reaction mixtures as described in MATERIALS AND METHODS, the amount of GAR produced in each case was determined as described, and the inhibitions were calculated. Each individual concentration of AMP (A) and GMP (B) was separately tested three to four times, and the curves represent a composite of the results. Symbols: ●, vegetative extract; □, myxospore extract.


A
AMP
B
GMP
% INHIBITION (GAR SYNTHESIS)
% INHIBITION (GAR SYNTHESIS)
100
80
60
40
20
5
10
15
20
25
NUCLEOTIDE (mM)

of the vegetative form of the enzymes, whereas the open-square curves depict inhibition of the myxospore form of the enzymes.

As illustrated in Fig. 9, the vegetative amidotransferase is inhibited to about the same extent by various concentrations of either AMP or GMP, and the inhibition by both is rather tight compared with the AMP-GMP control of the vegetative aminotransferase (Fig. 10). In fact, the aminotransferase inhibition by GMP is very minimal even at best (high concentration), and the inhibition by AMP, although more severe, is noticeably less strict than in the case of the amidotransferase.

By replotting the data in Fig. 9 in the form of Taketa and Pogell (146), n' values were obtained for AMP and GMP. Both gave an n' value for the amidotransferase of 2.2, indicating a cooperative homotropic effect between the molecules of these two nucleotides. By replotting the data of Fig. 10 for AMP in a similar fashion, an n' value of 1.36 was obtained for the aminotransferase.

Amido- and Aminotranferases of Myxospores (CW-2)

It was of special importance to examine M. xanthus myxospores for PP-ribose-P amidotransferase and ribose-5-phosphate aminotransferase because: (i) no one had reported their presence in any type of prokaryotic spore (purine salvage and interconversion enzymes, however, have been found in endospores) (71), and (ii) there is some indication (52) that myxospore formation is triggered by the onset or termination of some purine metabolic reaction(s).

To determine the presence or absence of the two enzymes in myxospores, extracts from 2.5-h myxospores were assayed (assays 1 and 1a) for amido- and aminotransferase activities. Both of the enzymes were found to be present in such myxospores. By employing assay 1, fresh vegetative extract catalyzed the amidotransferase-dependent formation of 0.69 nmol of GAR per mg of protein per min versus 0.56 nmol of GAR per mg of protein per min by fresh myxospore extract. By employing assay 1a, fresh vegetative extract catalyzed the aminotransferase-dependent formation of 0.19 nmol of GAR per mg of protein per min versus 0.20 nmol of GAR per mg of protein per min by fresh myxospore extract. If it is assumed that little or no catalytic damage to the myxospore enzymes resulted from the extra sonication necessary for extract preparation and that the extracts contained saturating levels of GAR synthetase (99, 113, 114), then it can be concluded from these results that similar levels of the amidotransferase and aminotransferase per milligram of protein exist in vegetative cells and myxospores. It would be incorrect to infer that the specific activities given above on a per milligram of protein basis are equivalent to activities on a per cell basis because myxospores have 35% more protein than vegetative cells (24).

To test for an allosteric alteration of the two enzymes that might have occurred as a consequence of sporulation, both vegetative and myxospore extracts were assayed (assays 1 and 1a) for amido- and aminotransferase activities in the presence of various levels of AMP and GMP. As indicated in Fig. 9 and Fig. 10, little or no change in the

enzymes' dose response to AMP or GMP resulted from myxospore morphogenesis, suggesting that the two enzymes' feedback inhibition response and, hence, their AMP-GMP dependent, allosteric structure is changed little by sporulation. The greatest variation in inhibitory response occurred in the case of the aminotransferase at 25 mM GMP (Fig. 10, graph B). The difference in inhibition between the vegetative and myxospore form of the enzyme at that GMP concentration (48 versus 15%) may or may not be significant. Repeated runs gave varying results, and the points on the graph represent the averages of five to six runs. Such variation did not occur at lower concentrations of GMP.

Effect of Cyclic AMP on the Amido- and Aminotransferase of M. xanthus, CW-2

In slime molds (69), both the intra- and the extracellular levels of cyclic AMP appear to be important in controlling morphogenesis. High levels allow the synthesis of certain enzymes and permit cellular aggregation, whereas low levels restrict enzyme synthesis and prevent cellular aggregation. It seemed possible that this also might be the case in M. xanthus because cyclic AMP enhances fruiting body formation in this organism (14). The possibility was entertained that high levels of cyclic AMP might be produced through a relaxation of feedback inhibition of the myxospore amido- and aminotransferase. This idea was checked by examining the vegetative and myxospore amido- and aminotransferases for their response to cyclic AMP inhibition (Table 4). The vegetative and the myxospore amido- and aminotransferases showed

little or no difference in their moderate degree of inhibition by cyclic AMP.

Older Myxospore of M. xanthus, CW-2

Older, 8-h myxospores, which are more biochemically mature than their 2.5-h counterparts (24), were checked for the amido- and amino-transferases and both enzymes were detected at roughly the same level in both types of spores. It was also noted that both the amido- and aminotransferases from the older myxospores are inhibited by AMP, GMP, and cyclic AMP to approximately the same extent as their counterparts from the younger (2.5 h) myxospores.

In Vivo Purine-Thiamine De Novo Synthesis: Glycine Uptake Studies

The finding of both the amido- and aminotransferases in myxospore extracts immediately raised the question of whether these two enzymes actually operate *in vivo* within the dormant and partially dehydrated myxospore. It was considered possible that conditions within the myxospore may have been unsuitable enough to temporarily render the two enzymes (as well as other *de novo* enzymes) partially or completely non-functional.

To answer the question concerning *de novo* synthesis in the myxospore, a two thrusted approach was embarked upon in the laboratory. The first approach was to feed intact myxospores only partially

repressed of the purine de novo pathway (possibly other de novo pathways also partially repressed) with saturating levels of a radioactively labelled de novo precursor (i.e. glycine-2-^{14}C), observe the rate of uptake and compare this uptake rate to that of fully repressed myxospores. The rationale was that if the de novo enzymes were indeed operative in the partially repressed myxospores then they should be able to channel some of the available glycine into de novo synthesis whereas in the fully repressed cells, where de novo synthesis would be completely stopped, the de novo outlet would be completely unavailable for glycine usage.

The flow of glycine in the fully repressed myxospores might then back up (assuming little or no increased intracellular pooling of this amino acid and that the uptake step itself was not per se the rate limiting reaction of glycine metabolism) forcing a slow down in the rate of glycine uptake as compared to the myxospores only partially repressed where the slow down would not occur. An increased uptake of glycine by partially repressed myxospores could then be explained as the consequence of added glycine usage in purine nucleotide de novo synthesis (although glycine usage for other de novo synthesis could not be entirely excluded).

Fully and partially repressed vegetative cells and germinating myxospores were also compared for their rates of glycine uptake in order to observe whether de novo synthesis could be detected in the vegetative and germinating states of the organism as well. While there was uncertainty about whether myxospores would carry out purine de novo

synthesis it seemed very likely that both vegetative and germinating cells partially repressed would carry out this process (45). Any increased glycine uptake by partially repressed vegetative or germinating cells therefore would signal this capability and these cell systems could serve as positive controls.

A glycine-2-^{14}C uptake study of the design described above was originally conducted with strain CW-2 in the chemically defined medium of Witkin and Rosenberg (160) augmented with excess adenine (100 µg/ml) (fully repression of de novo enzymes) or without adenine (partially repression). CW-2, however, displayed extremely variable generation times (10 to 15 h) or no growth in this medium. For this reason, strain CW-2 was replaced by strain CW-1 and the chemically defined, synthetic medium was replaced by the complex dialyzed and undialyzed CT media described in MATERIALS AND METHODS. The changes produced an immediate and reproducible shortening of the generation time of M. xanthus vegetative cells (4.5-6 h), the exact time depending upon whether the medium was dialyzed or undialyzed.

Dialyzed CT medium (lacking any exogenous purine bases, nucleosides, and nucleotides) was used to obtain or maintain partially repressed vegetative cells, myxospores and germinating myxospores. Undialyzed CT-adenine medium (containing excess adenine) on the other hand was used to obtain or maintain fully repressed vegetative cells, myxospores and germinating myxospores. The details are described in MATERIALS AND METHODS.

The results of a glycine-2-^{14}C uptake study employing vegetative cells and myxospores obtained from dialyzed and undialyzed CT media are indicated in Fig. 11. Fully repressed myxospores at various stages of maturation (1 h, immature; 2.5 h, morphologically mature, and 8 h, physiologically and morphologically mature) as well as fully repressed vegetative cells and germinating myxospores (8 h) are compared with their partially repressed counterparts. The results indicate that all of the cell types, irrespective of the extent of purine de novo repression, have the capacity to take up glycine. Furthermore, it is apparent from the same results (Fig. 11) that partially repressed vegetative cells, myxospores and germinating myxospores all take up glycine at a more or less faster rate (at least over first 48 min period) than their fully repressed counterparts. This suggests, although not unequivocally, that partially repressed vegetative cells, myxospores and germinating cells can de novo synthesize purine nucleotides. The reason for the decline in glycine uptake in 1 and 2.5 h partially repressed myxospore after 24-48 min is not understood. All other cell types, including both fully and partially repressed forms, displayed an essentially linear uptake of glycine over a 120 min span.

So as to see how 1, 2.5 and 8 h myxospores compare to vegetative and germinating cells for glycine uptake the data in Fig. 11 were replotted. Fig. 12 shows this replotting, Fig. 12(a) depicts the uptake of fully repressed cell types and Fig. 12(b) shows the uptake of partially repressed cell types. The initial rate's of glycine-2-^{14}C uptake (for first 30 min) by the different cell types were also

Fig. 11. Glycine uptake by M. xanthus CW-1 cells grown in dialyzed CT medium (0000) and CT medium + adenine (100 µg/ml) (XXXX). Glycine uptake assays were performed as indicated in MATERIALS AND METHODS. External, radioactive glycine was added to a final concentration of 1 µCi/ml. The graphs were rendered by a programmed minicomputer-teletype.

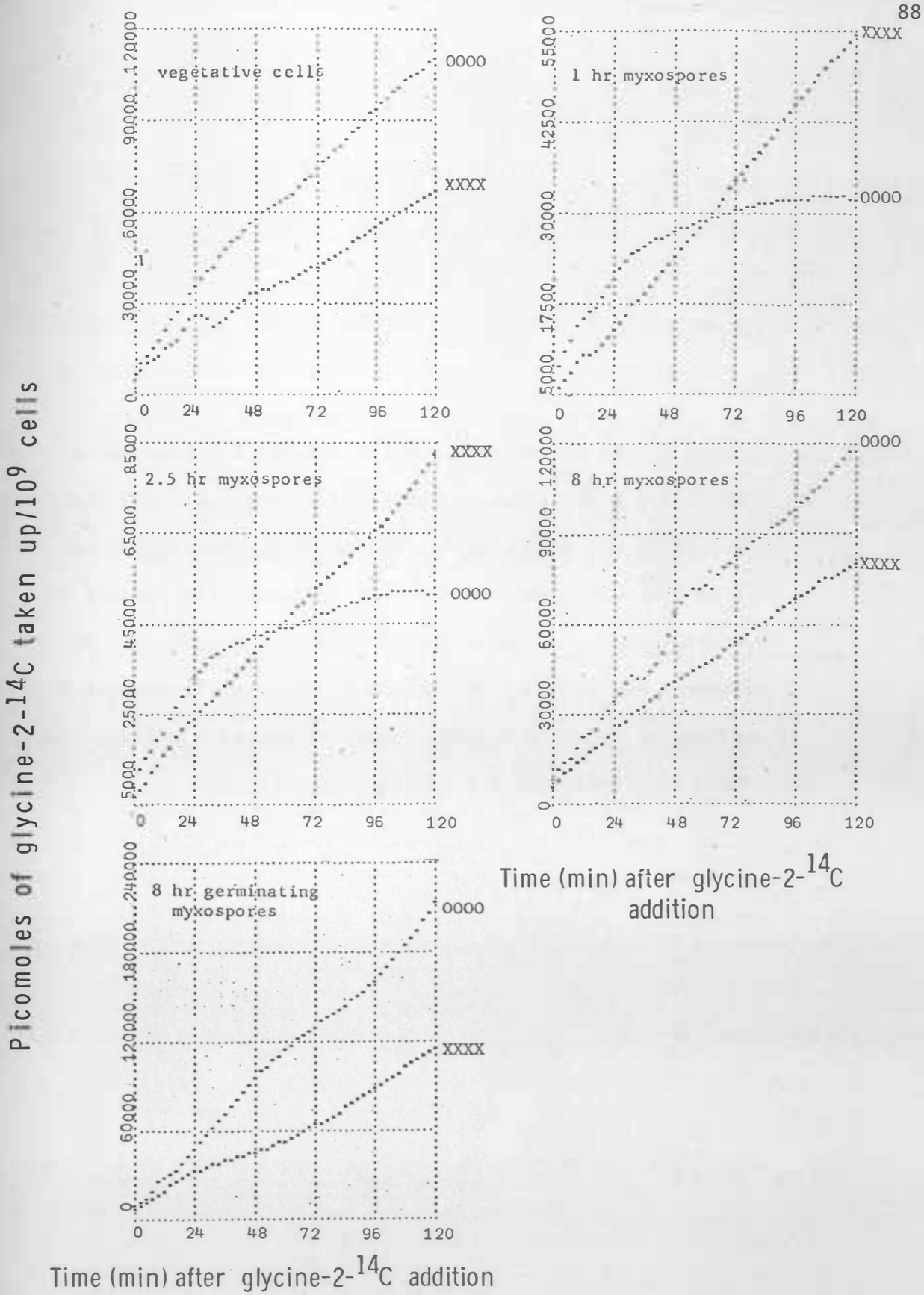

vegetative cells
0000
XXXX
0 30000 60000 90000 120000
0 24 48 72 96 120
1 hr myxospores
XXXX
0000
5000 17500 30000 42500 55000
0 24 48 72 96 120
2.5 hr myxospores
XXXX
0000
5000 25000 45000 65000 85000
0 24 48 72 96 120
8 hr myxospores
0000
XXXX
0 30000 60000 90000 120000
0 24 48 72 96 120
8 hr germinating myxospores
0000
XXXX
0 60000 120000 180000 240000
0 24 48 72 96 120
Picomoles of glycine-2-14C taken up/10^9 cells
Time (min) after glycine-2-14C addition
Time (min) after glycine-2-14C addition

Fig. 12. Glycine uptake by vegetative cells (AAAA), 1 h myxospores (BBBB), 2.5 h myxospores (CCCC), 8 h myxospores (DDDD) and germinating 8 h myxospores (EEEE) of M. xanthus CW-1 grown and/or maintained on (a) CT medium + adenine (100 µg/ml) or (b) dialyzed CT. Glycine uptake assays were performed as indicated in MATERIALS AND METHODS. External, radioactive glycine was added to a final concentration of 1 µCi/ml. The graphs were rendered by a minicomputer-teletype.

Picomoles of glycine-2-14C taken up/10^9 cells
0
30,000
60,000
90,000
120,000
EEEE
CCCC
DDDD
AAAA
BBBB
0
24
48
72
96
120
(a)
0
30,000
60,000
90,000
1 0,000
EEEE
DDDD
AAAA
CCCC
BBBB
0
24
48
72
96
120
(b)
Time (min) after glycine - 2 - 14C addition

calculated (from the plots) so as to further provide a clear comparison of the different cell types. The rates are indicated in Table 5. From both Fig. 11 and Table 5, it is apparent that the rate of glycine-2-^{14}C uptake during the first hour of myxospore induction drops off by about 50% and then returns back to the levels of the vegetative cell after completion of myxospore formation (2.5 h and 8 h). Germinating, 8 h myxospores (both fully and partially repressed) display the greatest rate of glycine-2-^{14}C uptake, even greater than that of vegetative cells.

In Vivo Purine-Thiamine De Novo Synthesis: TLC-Radioautographic Studies

The second approach that was used to detect de novo purine synthesis in the myxospore involved (i) extracting purine nucleotide pools from partially-repressed glycine-2-^{14}C fed myxospores (1 h, 2.5 h, and 8 h) and (ii) identifying the extracted and possibly labelled purines by thin layer chromatographic (TLC) and radioautographic analysis. The detection of any radioactive (from radioautography) purines (from Rf and UV coloration on two dimensional thin layer chromatograms) in a myxospore extract, it was reasoned, would indicate that purine de novo synthesis indeed functioned in the myxospore. The assumption was that the only way purine nucleotides could be labelled by glycine-2-^{14}C was by the de novo route (incorporation of glycine into purine nucleotides by a salvage route was consiered unlikely, see DISCUSSION for details).

Table 5. Initial rates of glycine, adenine and guanine uptake by *M. xanthus* CW-1. The rates were determined from polynomial regression analysis (on Hewlett-Packard minicomputer) of 0-30 min slopes of Fig. 11, 21 and 22.

Cell types	Initial rate of compound uptake[a] (picomoles/min/10^9 cells)					
	Glycine		Adenine		Guanine	
	CT + adenine	Dialyzed CT	CT + adenine	Dialyzed CT	CT + adenine	Dialyzed CT
Vegetative	649	1138	32	105	37	3
1 h myxospore	321	552	122	113	23	20
2.5 h myxospore	712	911	129	169	19	13
8 h myxospore	744	1114	81	93	62	35
Germinating 8 h myxospore	1016	1695	279	188	87	214

[a]For first 30 min of exposure.

The details of myxospore preparation, extraction, thin layer chromatography and radioautography are indicated in MATERIALS AND METHODS. Vegetative and germinating cells were also employed in this study and the details of their preparation and extraction are likewise indicated in MATERIALS AND METHODS. Extracts from partially-repressed, glycine-2-^{14}C fed vegetative and germinating cells were analyzed alongside the myxospore extracts by the TLC-radioautographic procedure. Vegetative and germinating cell extracts were considered to be positive controls and it was regarded as very likely that TLC nucleotide spots from these extracts would be radioactive.

Fig. 13 shows the typical pattern that was obtained after TL plates had been spotted with extracts from myxospores (1 h, 2.5 h and 8 h), vegetative cells and germinating cells (8 h myxospores) and chromatographed in the first dimension (a composite of several plates is shown). As can be seen in the composite radioautogram of Fig. 13, an unidentified UV absorbing spot with an Rf of about 0.78 occurred in the chromatogram of each extract. Each of these UV absorbing spots was found upon close examination to be composed of one upper dark-bluish, round portion and one lower light-bluish, shiny, crescent-like portion. The distribution of radioactivity between these two sectors varied from one spot to another and the quantitative differences in labelling of each of the spots (from vegetative cells, myxospores and germinating myxospores) is hard to evaluate.

In an attempt to determine the chemical nature of the unidentified UV-absorbing spot, a number of UV absorbing compounds of known identity

Fig. 13. One-dimensional, thin-layer chromatographic-radioautographic analysis of M. xanthus CW-1 myxospore, vegetative and germinating cell extracts and thiamine-HCl. Symbols: 1, thiamine-HCl; 2, vegetative cell; 3, 1 h myxospore; 4, 2.5 h myxospore; 5, 8 h myxospore; 6, germinating 8 h myxospore; 7, 2.5 h myxospore + thiamine-HCl. Abbreviations: A, UV absorbing spot; S, UV shiny spot. Partially repressed myxospores, vegetative cells and germinating cells were given glycine-2-^{14}C for 50-90 min and then were extracted according to the procedure in MATERIALS AND METHODS. 10-30 µl of each cell extract, and 5 mM thiamine-HCl were applied separately to the bottom of a thin layer plate and the plate was then chromatographed in the first dimension (n-butanol:acetone: diethylamine:water, 10:10:2:5) and UV-radioautographically analyzed as described in MATERIALS AND METHODS.

A
S
A
A
S
A
S
A
S
A
A
S
1
2
3
4
5
6
7

were chromatographed (alone or mixed with extract) in the same way and their Rf were compared to that of the unknown. The results are indicated in Fig. 13 and Table 6. As indicated in Table 6, the Rf value of thiamine-HCl is the only one close or similar in UV absorbance to that of the unidentified spot. The unidentified spot has an Rf of 0.78 and thiamine-HCl has an Rf of 0.79. Tyrosine which also had an Rf at 0.78 and absorbs UV light, has a light-gray UV coloration while the unidentified spot and thiamine-HCl both display dark UV colorations. The thiamine-HCl spot, like the unidentified spot, had two distinguishing sectors, one with a UV bluish and the other with a UV shiny coloration. When thiamine-HCl was cochromatographed with myxospore extract (8 h), the 0.78 Rf, UV-absorbing spot and authentic thiamine-HCl, completely overlapped. The results suggest that the unknown material may be thiamine-HCl or a close relative, however, further studies are required to confirm this assumption.

Besides separating the thiamine-like substance from the rest of the cellular (myxospore, vegetative and germinating) components, the TLC against n-butanol:acetone:diethylamine:water (10:10:2:5) also was effective in moving amino acids (including glycine) away from the origin while retaining purine nucleotides (also pyrimidine nucleotides and thiamine pyrophosphate) at or near the starting mark (Table 6). This was important so that any radioactivity of purine nucleotides would not be masked by that of glycine-2-^{14}C or any other radioactive amino acids.

In order to separate purine nucleotides from other radioactive substances which may have also remained at the origin after first

Table 6. Rf values of unidentified UV absorbing substance(s) in myxospore extract, authentic purines, pyrimidines, thiamine and related compounds thin layer chromatographed in first dimension against n-butanol:acetone:diethylamine:water, 10:10:2:5.

Substance(s)[a]	Rf[b]
Thiamine pyrophosphate (cocarboxylase)	0.26
S-adenosyl-methionine	0.41
AMP	0.13
ADP	0.05
ATP	0.07
GMP	0.30
GDP	0.22
GTP	0.25
Mixture of CMP, CDP, CTP	0.33[c]
Mixture of UMP, UDP, UTP	0.31[c]
Mixture of cytidine and uridine	0.55[c]
cyclic AMP	0.59
Thiamine-HCl	0.79
Xanthine	0.50
Histidine	0.37[e]
Tryptophan	0.74[e]
Phenylalanine	0.67[e]
Tyrosine	0.78[e]
Mixture of adenine and guanine	0.63[c]
Mixture of adenosine and guanosine	0.63 and 0.50
Glycine-2-^{14}C	0.43[d]
Unidentified UV-absorbing spot in 8 h myxospore extract	0.78
8 h myxospore extract + thiamine-HCl	0.85[c]

[a]10 µl of compound (5 mM) was spotted individually on a thin layer chromatoplate as described in MATERIALS AND METHODS. Each plate was one-dimensionally chromatographed against n-butanol:acetone:diethylamine: water (10:10:2:5). The movement of all chromatographed substances and their Rf's were determined by the UV absorbance of the materials.

[b]The Rf value of each compound was calculated from the distance traveled by the substance from the origin divided by the distance traveled by the solvent front.

[c]Only one discernible spot.

[d]By radioautography.

[e]Light-gray in color under UV-light.

dimensional TLC, a chromatography in the second dimension was carried out on the chromatograms. The solvent system was composed of 1.5 M potassium phosphate buffer (pH 3.4) which separates nucleotides on the basis of their charge (118). Most nucleotides tended to migrate up near the solvent front and form spots at this location. Individual nucleotide spots (usually not completely separated from other nucleotide spots) were identified by their UV absorbance and characteristic RF values but in some cases also by their distinctive UV coloration (guanine nucleotides gave light blue spots under UV light). The RF values of known, bona fide nucleotides in this two dimensional system were determined by two dimensional chromatography of authentic samples run either by themselves or mixed with cellular extracts and run as a mixture. The known samples, whatever their composition, were treated in an identical way to test extracts.

The results of a two-dimensional TLC-radioautographic analysis of myxospore (1, 2.5, and 8 h), vegetative and germinating (8 h myxospore) cell extracts are shown in Figs. 14-18. Partially repressed cells which had been given glycine-2-^{14}C over a 50-90 min time interval were employed to make these extracts. Also indicated in the Fig. 14-18 are the chromatographic behaviors of known nucleotides and thiamine derivatives which were cochromatographed with certain cellular extracts. The chromatographic behaviors of known nucleotides was used to deduce the identity of unknown cellular nucleotides.

Most of the chromatograms revealed a dark UV absorbing spot directly above the origin near the solvent front (Figs. 14 a, c, d;

Fig. 14. Two-dimensional thin-layer chromatography-radioautography of extract of partially-repressed, vegetative cells (CW-1) given glycine-2-^{14}C for 90 min. Vegetative cells were grown, administrated glycine-2-^{14}C and extracted as indicated in MATERIALS AND METHODS. Thin layer plates were spotted with vegetative cell extract or extract mixed with a known substance and then were developed in two dimensions as indicated in MATERIALS AND METHODS. The chromatograms were exposed to X-ray film for 7 days for radioautography. Symbols: (a), 30 µl of vegetative cell extract; (b) 10 µl of vegetative cell extract + 10 µl of 5 mM ATP; (c), 10 µl of vegetative cell extract + 10 µl of 5 mM GTP; (d), 10 µl of vegetative cell extract + 10 µl of 5 mM thiamine-HCl.

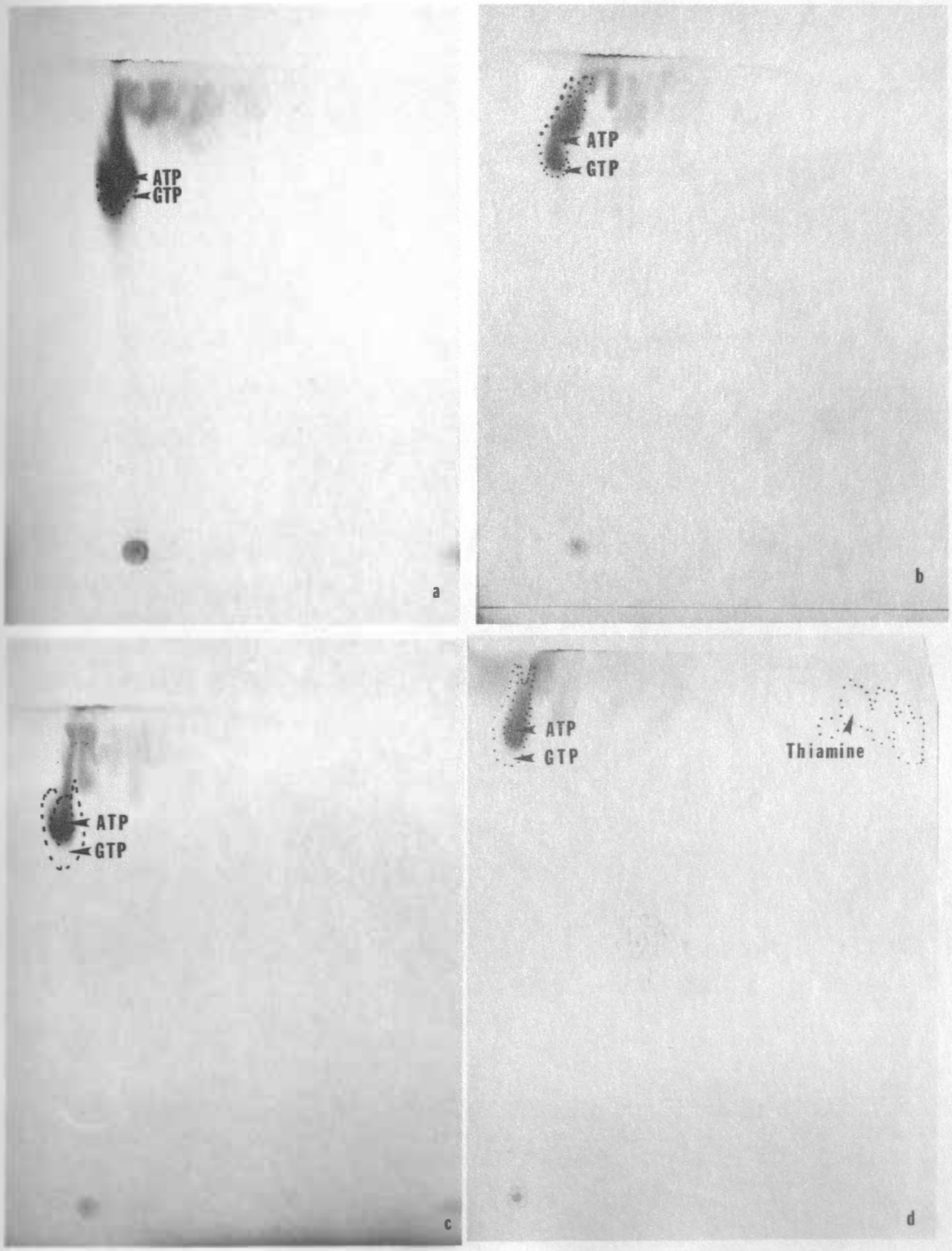

ATP
GTP
a
ATP
GTP
b
ATP
GTP
c
ATP
GTP
Thiamine
d

Fig. 15. Two-dimensional thin-layer chromatography-radioautography of extract of partially repressed 1 h myxospores (CW-1) given glycine-2-^{14}C for 90 min. Myxospores (1 h) were prepared, administered glycine-2-^{14}C and extracted as indicated in MATERIALS AND METHODS. Thin layer plates were spotted with 1 h myxospore extract or extract mixed with a known substance and then were developed in two dimensions as indicated in MATERIALS AND METHODS. The chromatograms were exposed to X-ray film for 7 days for radioautography. Symbols: (a), 30 µl of 1 h myxospore extract; (b), 10 µl of 1 h myxospore extract + 10 µl of 5 mM AMP; (c), 10 µl of 1 h myxospore extract + 10 µl of 5 mM GMP; (d), 10 µl of 1 h myxospore extract + 10 µl of 5 mM GTP.

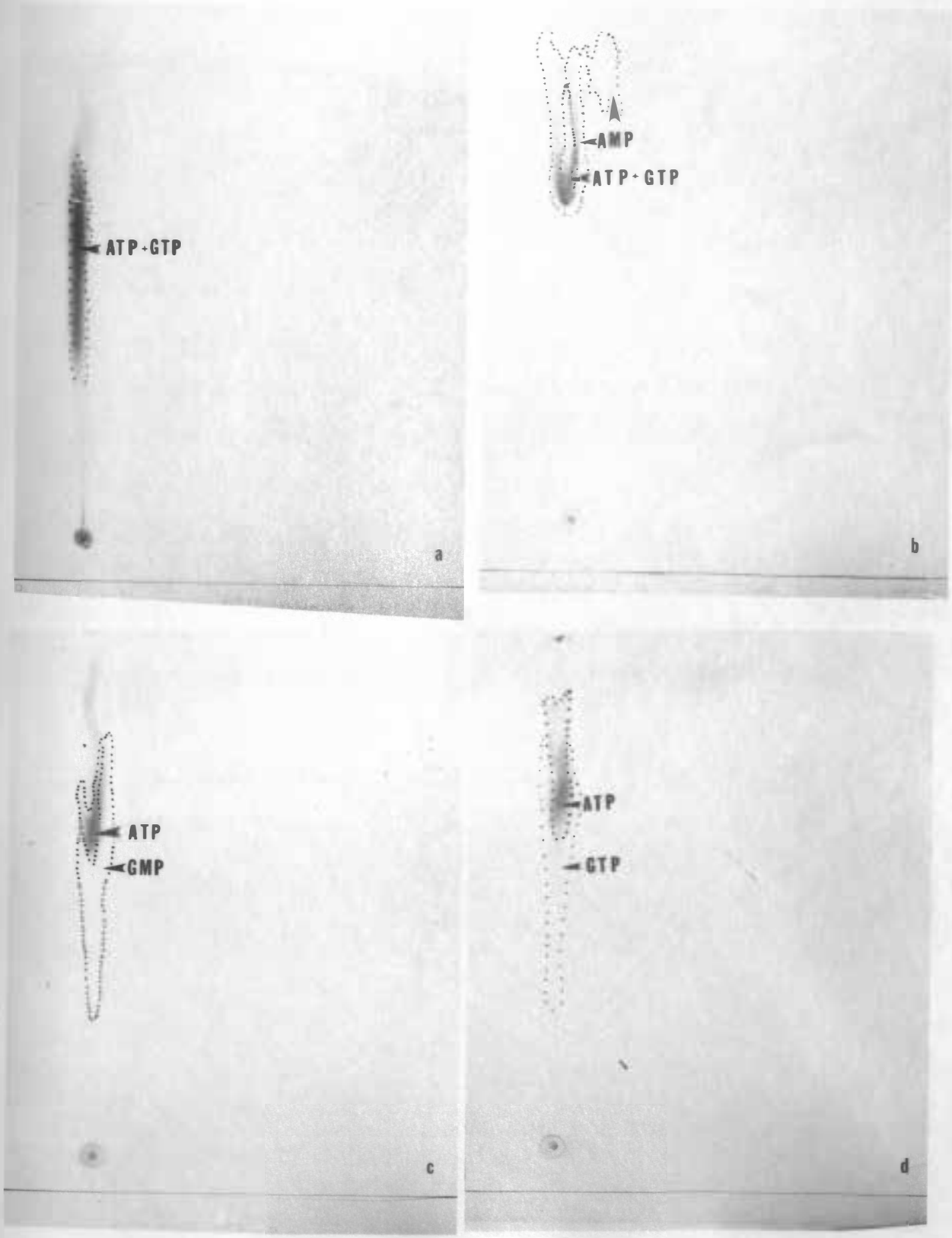

ATP+GTP
a
AMP
ATP+GTP
b
ATP
GMP
c
ATP
GTP
d

Fig. 16. Two-dimensional thin-layer chromatography-radioautography of extract of partially repressed 2.5 h myxospores (CW-1) given glycine-2-^{14}C for 90 min. Myxospores (2.5 h) were prepared, administered glycine-2-^{14}C and extracted as indicated in MATERIALS AND METHODS. Thin layer plates were spotted with 2.5 h myxospore extract or extract mixed with a known substance and then were developed in two dimensions as indicated in MATERIALS AND METHODS. The chromatograms were exposed to X-ray film for 7 days for radioautography. Symbols: (a) 10 µl of 2.5 h myxospore extract; (b) 10 µl of 2.5 h myxospore extract + 10 µl of 5 mM AMP; (c) 10 µl of 2.5 h myxospore extract + 10 µl of 5 mM thiamine-HCL; (d), 10 µl of 2.5 h myxospore extract + 10 µl of 5 mM CMP.

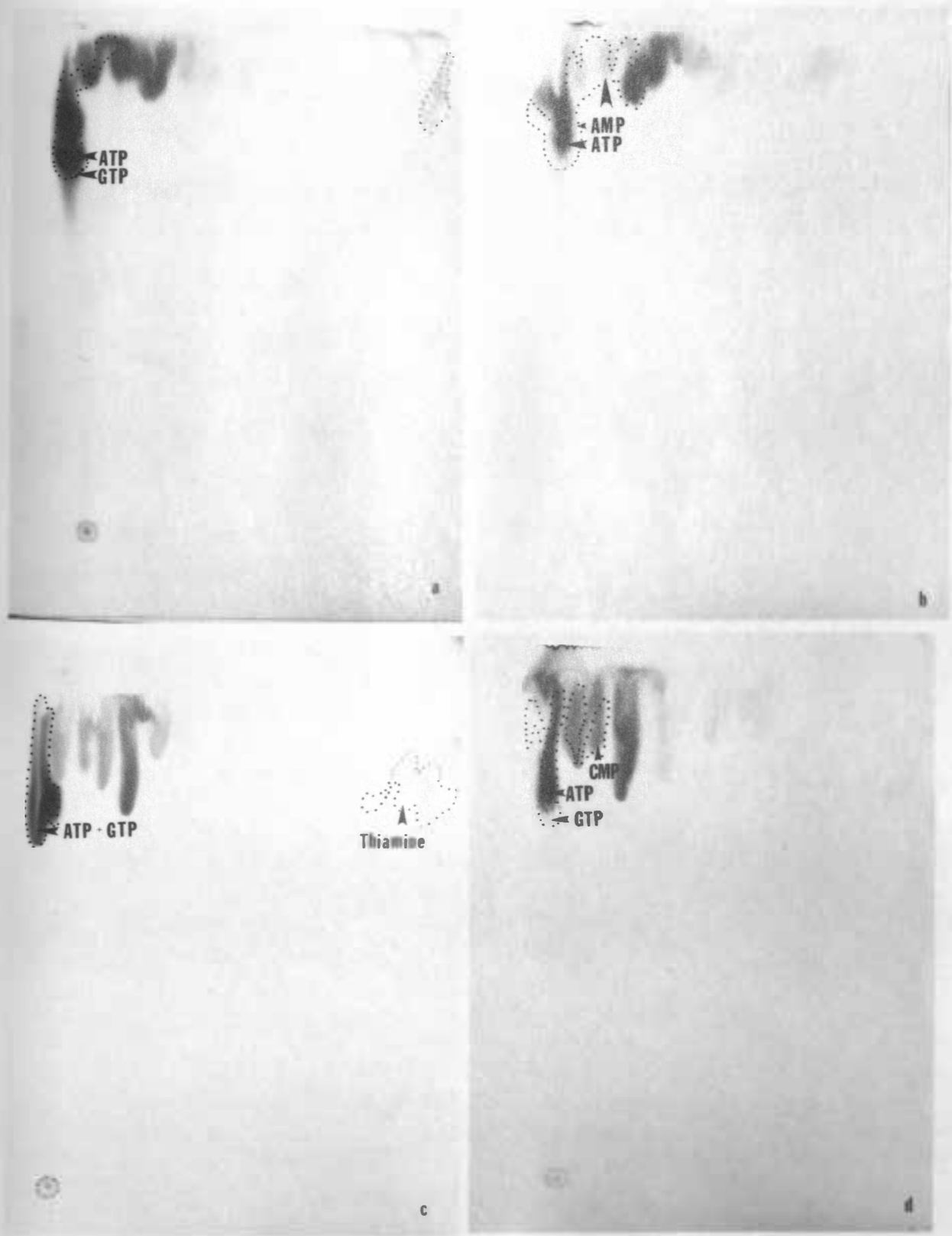
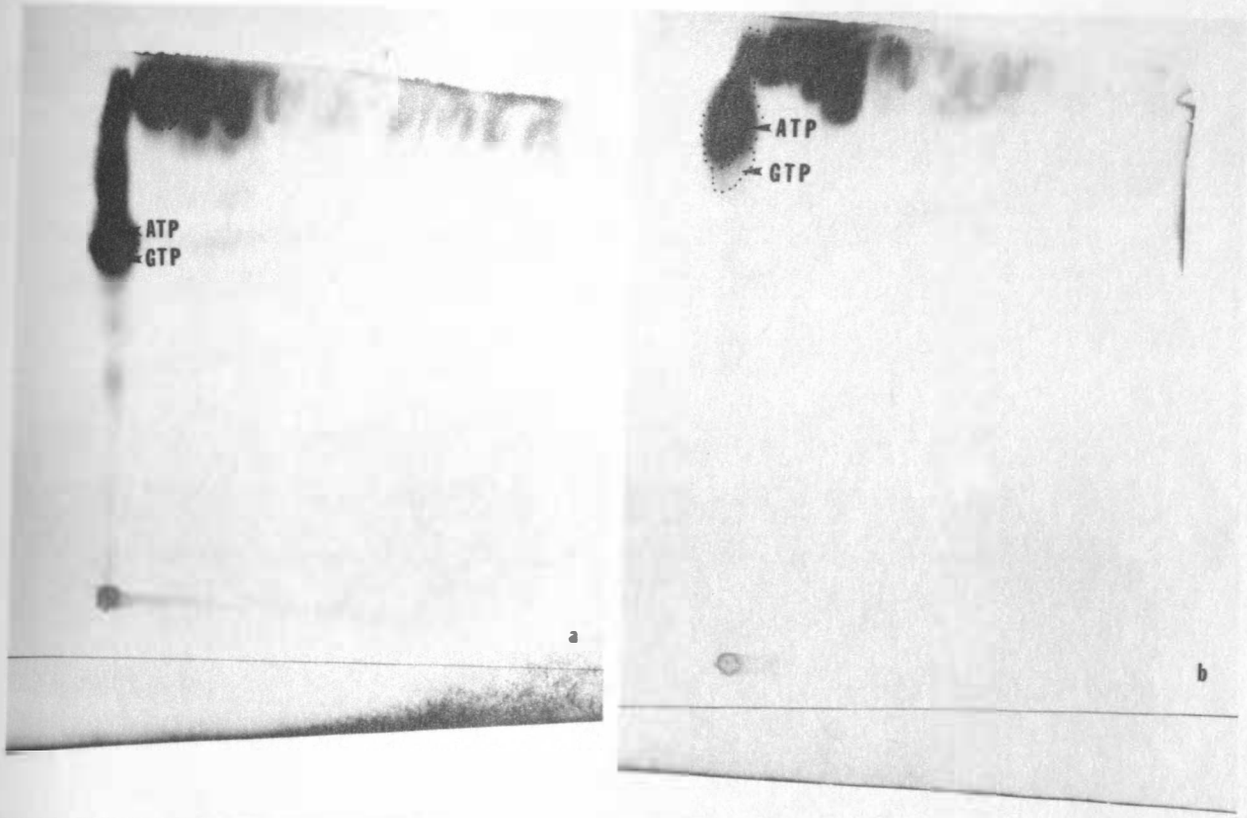

ATP
GTP
a
AMP
ATP
b
ATP · GTP
Thiamine
c
CMP
ATP
GTP
d

Fig. 17. Two-dimensional thin-layer chromatography-radioautography of extract of partially repressed 8 h myxospores (CW-1) given glycine-2-^{14}C for 90 min. Myxospores (8 h) were prepared, administered glycine-2-^{14}C and extracted as indicated in MATERIALS AND METHODS. Thin layer plates were spotted with 8 h myxospore extract or extract mixed with a known substance and then were developed in two dimensions also as indicated in MATERIALS AND METHODS. The chromatograms were exposed to X-ray film for 7 days for radioautography. Symbols: (a) 10 µl of 8 h myxospore extract; (b) 10 µl of 8 h myxospore extract + 10 µl of 5 mM ATP; (c) 10 µl of 8 h myxospore extract + 10 µl of 5 mM GTP; (d) 10 µl of 8 h myxospore extract + 10 µl of 5 mM thiamine-HCl.


ATP
GTP
a
ATP
GTP
b

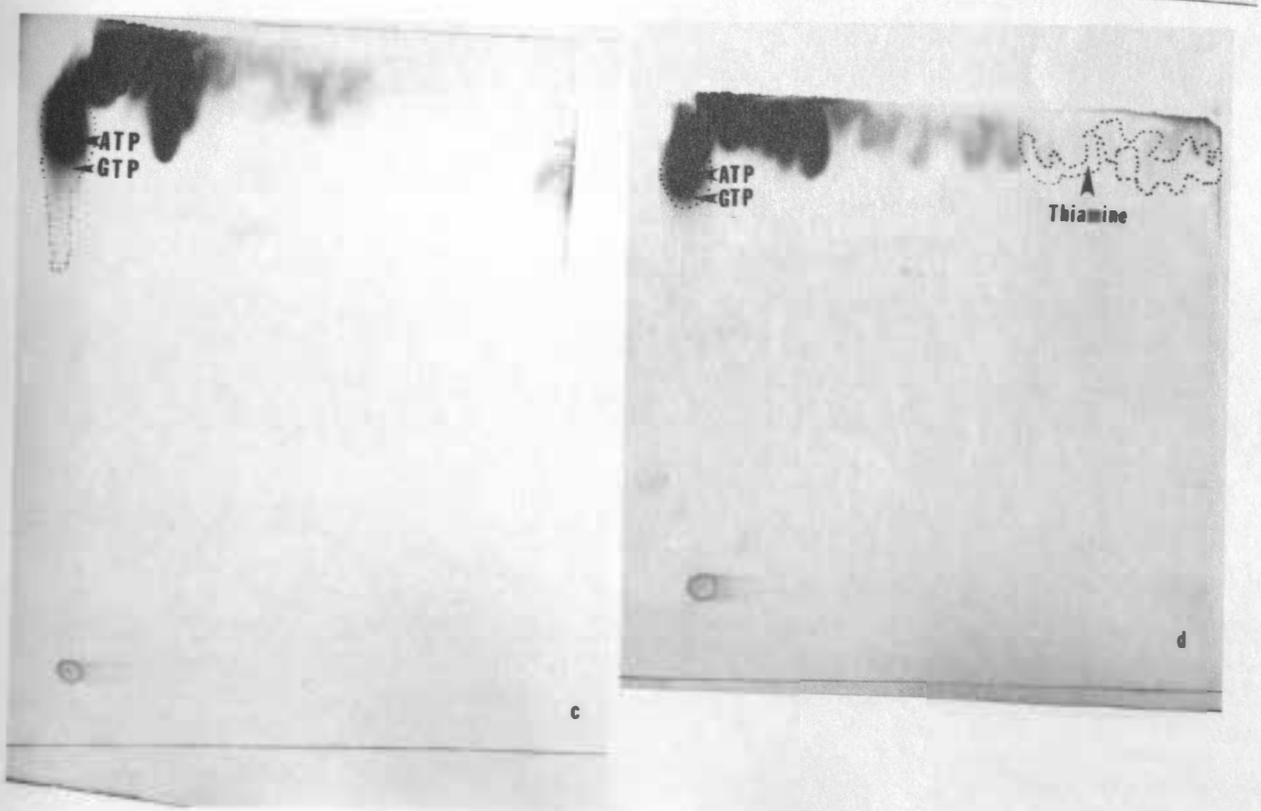
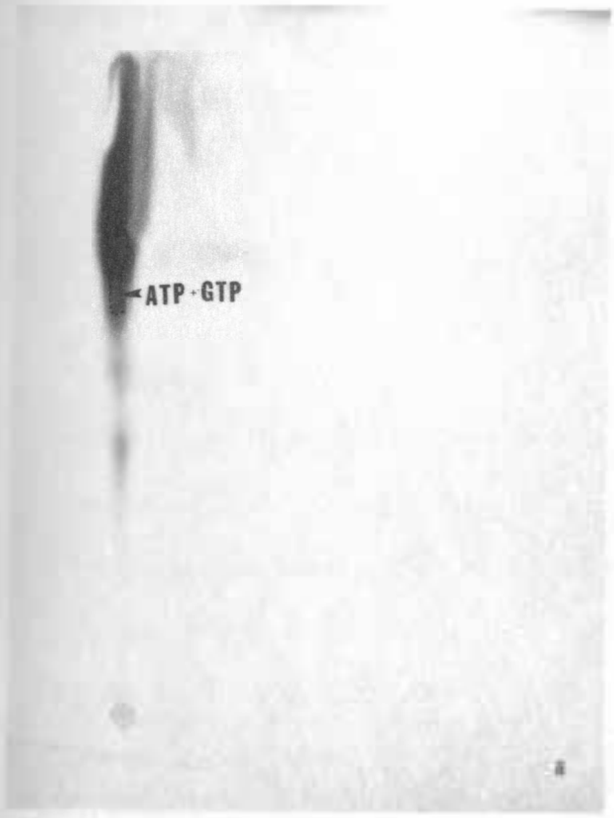
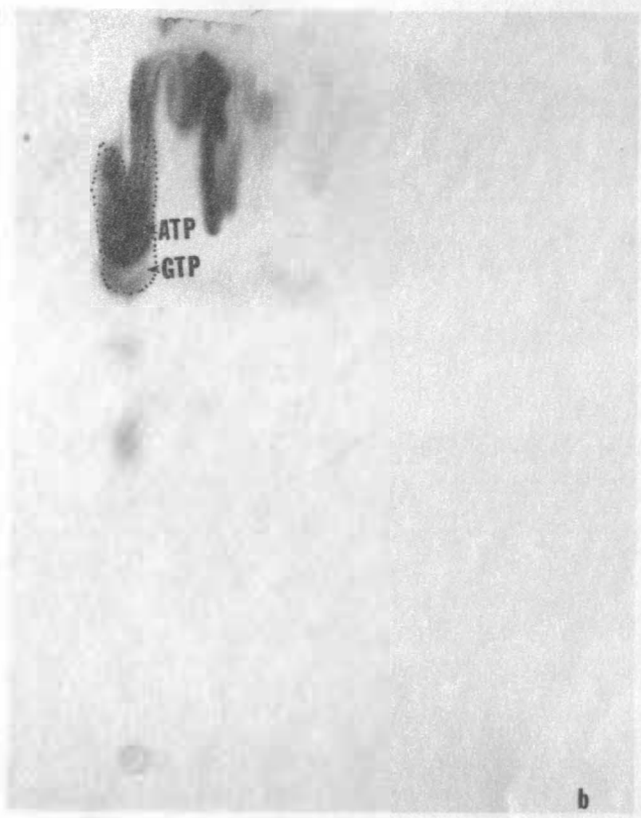
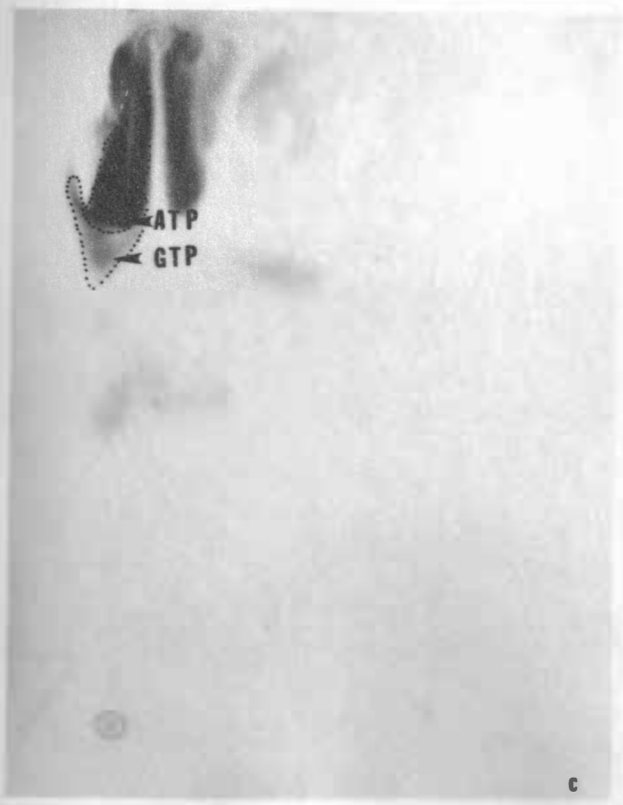

ATP
GTP
c
ATP
GTP
Thiamine
d

Fig. 18. Two-dimensional thin-layer chromatography-radioautography of extract of partially repressed germinating 8 h myxospores (CW-1) given glycine-2-^{14}C for 50 min. Germinating 8 h myxospores were prepared, administered glycine-2-^{14}C and extracted as indicated in MATERIALS AND METHODS. Thin layer plates were spotted with germinating 8 h myxospore extract or extract mixed with a known substance and then were developed in two dimensions as indicated in MATERIALS AND METHODS. The chromatograms were exposed to X-ray film for 7 days for radioautography. Symbols: (a) 10 µl of germinating 8 h myxospore extract; (b) 10 µl of germinating 8 h myxospore extract + 10 µl of 5 mM ATP; (c) 10 µl of germinating 8 h myxospore extract + 10 µl of 5 mM GTP; (d) 10 µl of germinating 8 h myxospore extract + 10 µl of 5 mM CMP.


ATP · GTP
a


ATP
GTP
b


ATP
GTP
c

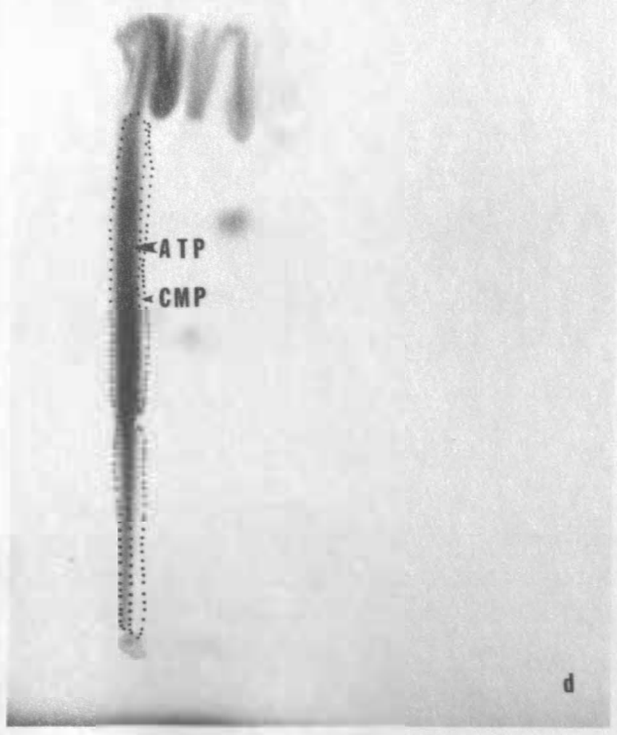
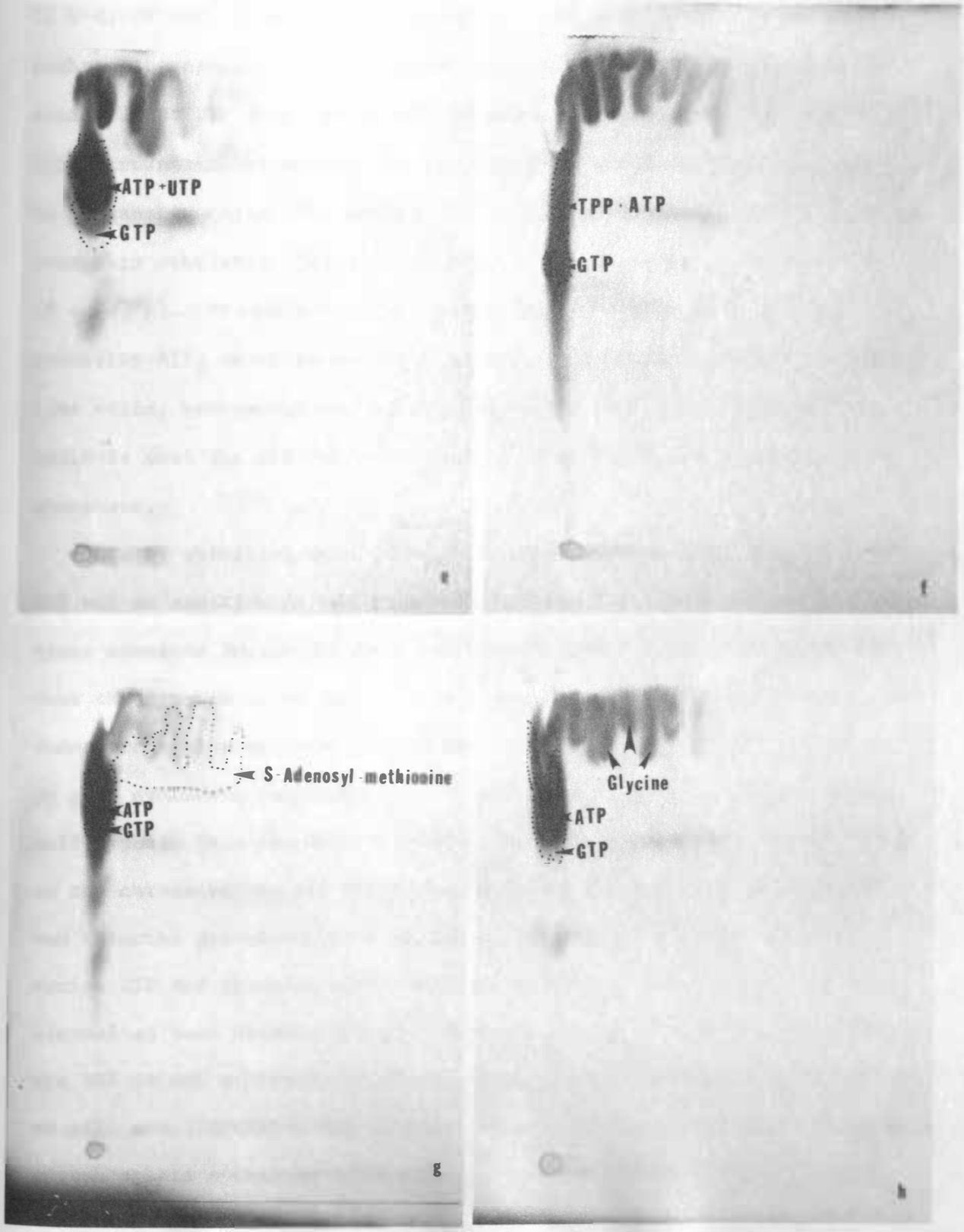

ATP
CMP
d

Fig. 18 (continued). (e) 10 µl of germinating 8 h myxospore extract + 10 µl of 5 mM UTP; (f) 10 µl of germinating 8 h myxospore extract + 10 µl of 5 mM TPP; (g) 10 µl of germinating 8 h myxospore extract + 10 µl of 5 mM S-adenosyl-methionine; (h) 10 µl of germinating 8 h myxospore extract + 5 µl of 0.06 mM glycine-2-^{14}C (specific activity of 5.93 µCi/mmole).


ATP+UTP
GTP
e
TPP·ATP
GTP
f
S-Adenosyl-methionine
ATP
GTP
g
Glycine
ATP
GTP
h

15 b-d; 16 a-d; 17 a, c, d; 18 c, g, h). This UV absorbing substance cochromatographed exactly like ATP (Figs. 14 b, 17 b, 18 b) and very similarly to UTP (Fig. 18 e) and thiamine pyrophosphate (Fig. 18 f). Other known nucleotides likely to occur in significant concentrations in M. xanthus cells (45) and thiamine-HCl cochromatographed differently from this substance (Figs. 14 c, d; 15 b, c, d; 16 b, c, d; 17 c, d; 18 c, d, g). The results suggest that the substance is wholly or primarily ATP, which is known to occur in significant amounts in vegetative cells, myxospores and germinating cells (45). The results also indicate that the ATP may be contaminated with UTP and thiamine pyrophosphate.

The UV absorbing spot on the chromatograms not only behaved like ATP but in addition it was intensely radioactive in all of the chromatograms examined including those of myxospore extracts. This indicates that the myxospore, as well as the vegetative and germinating cell, can incorporate glycine into ATP and supports the hypothesis that purine de novo synthesis functions in the myxospore as well as in the other cell types. This conclusion is not unequivocal, however, since the ATP on the chromatograms may have been contaminated with radioactive UTP and thiamine pyrophosphate. On the other hand, the levels of radioactive UTP and thiamine pyrophosphate in the ATP spots should be very minimal at best because glycine, the sole source of radioactivity for the UTP is not a direct precursor of UTP (and in fact very indirect, if at all, see DISCUSSION for details) as it is of ATP and because thiamine pyrophosphate occurs in cell extracts in only minute quantities (92).

Most of the chromatograms besides revealing an ATP spot also displayed a light-blue absorbing crescent-shaped spot immediately underneath the ATP spot (Figs. 14 a, b, d; 15 c; 16 a, d; 17 a, b, d; 18 b, e, g, h). The material in this spot chromatographed exactly like authentic GTP, which also produces a light-blue crescent-shaped spot in the two dimensional TLC system (Figs. 14 c; 15 d; 17 c; 18 c). The GTP spot in the chromatograms may have been contaminated by GMP which chromatographs in the same vicinity as GTP and also produces a light-blue UV absorbing spot (usually a trailing spot, however, is produced by GMP) (Fig. 15 c). Other nucleotides and thiamine derivatives chromatographed differently than GTP, although AMP had a tendency to overlap the GTP spot (Figs. 15 b; 16 b).

The GTP spot on all of the chromatograms was radioactive although less intensely so than the ATP spot (Figs. 14 a; 16 a; 17 a; 18 b, e, g, h). In addition, the radioactivity on the GTP spot was considerably diluted (as observed by radioautography) when authentic non-radioactive GTP was present (Figs. 14 c; 15 d; 17 c; 18 c) an effect that was not apparent with the ATP spot. The finding of radioactivity in the GTP of myxospores, vegetative cells and germinating cells reaffirms the earlier stated hypothesis that all cell types of M. xanthus can carry out purine de novo synthesis.

Certain extracts, for unknown reasons, allowed a mixing of the ATP and GTP during the two dimensional TLC (Figs. 15 a; 16 c; 18 a, d, f). This may have been caused in part by the different batches of TLC plates that were used in this study.

Purine Interconversion and Salvage: Adenine and Guanine Uptake Studies

It is well established that purine nucleotides can be synthesized either *de novo* or from externally provided purine bases or nucleosides (salvage pathway) in different biological systems, ranging from bacteria (5, 90, 166) to mammals (90). The first indication of purine interconversion and salvage in *M. xanthus* came from the findings of Hemphill and Zahler (51), who found that the purine bases, nucleosides, or nucleotides could support the growth of adenine- or guanine-requiring mutants.

An *in vivo* purine (adenine or guanine) uptake study was designed so as to determine the role of purine interconversion and salvage during the vegetative growth of *M. xanthus*, CW-1. In addition, a similar study was also designed for myxospores (1 h, 2.5 h and 8 h) and germinating cells (8 h myxospores) in order to explore the possible role played by purine interconversion and salvage utilization in the morphogenesis of this organism.

The various types of CW-1 cells used for the purine uptake study were grown and/or maintained under conditions similar to those used for the glycine-uptake study so that cells fully and partially repressed of purine *de novo* synthesis would be available. The various types of cells (both fully and partially repressed) were administered adenine-8-^{14}C or guanine-8-^{14}C and the rates of uptake of these purines were observed by the same filtration technique used in the glycine

uptake studies. The rational was that if any measurable uptake of exogenous purines occurred this would indicate that externally supplied purines could, in fact, be taken up by M. xanthus and presumably then converted to functional nucleotides. Differences in uptake of adenine or guanine between partially and fully repressed cells would provide some indication as to the inducible (or derepressible) nature of the enzymes (e.g. purine phosphoribosyltransferases) involved in the usage of external purine bases.

As indicated in Figs. 19 and 20, both fully and partially repressed myxospores, vegetative cells and germinating myxospores displayed a significant ability to take up exogenous adenine and guanine. Also apparent is the fact that, while many of the cell types (both fully and partially repressed) discontinued (some very early) their net uptake (turnover possible) of guanine (before the 120 min time period had fully elapsed), they did not discontinue, although in most cases they diminished, their uptake of adenine. It would appear from these results that both exogenous adenine and guanine play a role in the metabolism of M. xanthus but that quantitatively adenine is more important than guanine.

Interestingly, the most active cell type in taking up both adenine and guanine was the germinating myxospore and the least active, or among the least active, was the vegetative cell. Dormant, non-germinating myxospores also were generally more active in taking up adenine and guanine than vegetative cells (8 h myxospores took up adenine and guanine at least as fast as vegetative cells and, in all

Fig. 19. Adenine uptake by vegetative cells (AAAA), 1 h myxospores (BBBB), 2.5 h myxospores (CCCC), 8 h myxospores (DDDD) and germinating 8 h myxospores (EEEE) of M. xanthus CW-1. Symbols: (a), fully repressed cells; (b), partially repressed cells. Fully repressed vegetative cells were grown on CT + adenine (100 µg/ml) and fully repressed dormant and germinating myxospores were obtained from such vegetative cells and were maintained on CT medium or buffer containing 100 µg/ml of adenine. Partially repressed cells were similarily prepared except that they were grown and/or maintained on dialyzed CT medium containing no adenine. The details are described in MATERIALS AND METHODS. Adenine uptake assays were performed as indicated in MATERIALS AND METHODS. The graphs were rendered by a programmed minicomputer-teletype.

Picomoles of adenine-8-14C taken up/10^9 cells
17500
13500
9500
5500
1500
0
24
48
72
96
120
EEEE
CCCC
BBBB
DDDD
AAAA
(a)
17000
13000
9000
5000
1000
0
24
48
72
96
120
EEEE
CCCC
AAAA
DDDD
BBBB
(b)
Time (min) after adenine-8-14C addition

Fig. 20. Guanine uptake by vegetative cells (AAAA), 1 h myxospores (BBBB), 2.5 h myxospores (CCCC), 8 h myxospores (DDDD) and germinating 8 h myxospores (EEEE) of M. xanthus CW-1. Symbols: (a), fully repressed cells; (b), partially repressed cells. Fully repressed vegetative cells were grown on CT + guanine (100 µg/ml) and fully repressed dormant and germinating myxospores were obtained from such vegetative cells and were maintained on CT medium or buffer containing 100 µg/ml of guanine. Partially repressed cells were similarly prepared except that they were grown and/or maintained on dialyzed CT medium containing no guanine. The details are described in MATERIALS AND METHODS. Guanine uptake assays were performed as indicated in MATERIALS AND METHODS. The graphs were rendered by a programmed minicomputer-teletype.

Picomoles of guanine-8-14C taken up/10^9 cells
5700
4450
3200
1950
700
0
24
48
72
96
120
EEEE
DDDD
AAAA
CCCC
BBBB
(a)
9800
7550
5300
3050
800
EEEE
CCCC
DDDD
BBBB
AAAA
(b)
Time (min) after guanine-8-14C addition

but one case, actually faster than vegetative cells) and there was a tendency, although not always clear cut, for older (8 h) myxospores to be more active than younger, immature (1 h) myxospores. All of these results belie the supposed dormant state of the myxospore and bring into question the extent and scope of this dormancy (although part of explanation may lie in larger cell size of myxospore).

To compare the adenine and guanine uptake rates of fully to partially repressed cell types, the data of Figs. 19 and 20 were replotted and the initial rates of uptake were calculated. The results are shown in Figs. 21 and 22 and Table 5. One of the common features in both figures and in Table 5 is that there occurs no consistent difference in adenine or guanine uptake between fully and partially repressed cells.

A reduced initial rate of uptake of the purines by partially repressed cells or a significantly long lag before the onset of uptake, would have indicated that an inducible active-transport carrier (phosphoribosyltransferase) had to be synthesized before uptake could have commenced. The lack of any reduced uptake of the purines by partially repressed cells (exceptions are guanine uptake by vegetative cells and 8 h myxospores) and the absence of any significant lag in this uptake indicates that the carriers are probably constitutive (non-induced) proteins.

Fig. 21. Adenine uptake by vegetative cells, myxospores (1, 2.5 and 8 h) and germinating myxospores (8 h) of M. xanthus CW-1. Symbols: (OOOO), partially repressed cells; (XXXX), fully repressed cells. Partially and fully repressed cells were obtained and the adenine uptake assay was performed as indicated in Fig. 19 and MATERIALS AND METHODS. The graphs were rendered by a programmed minicomputer-teletype.

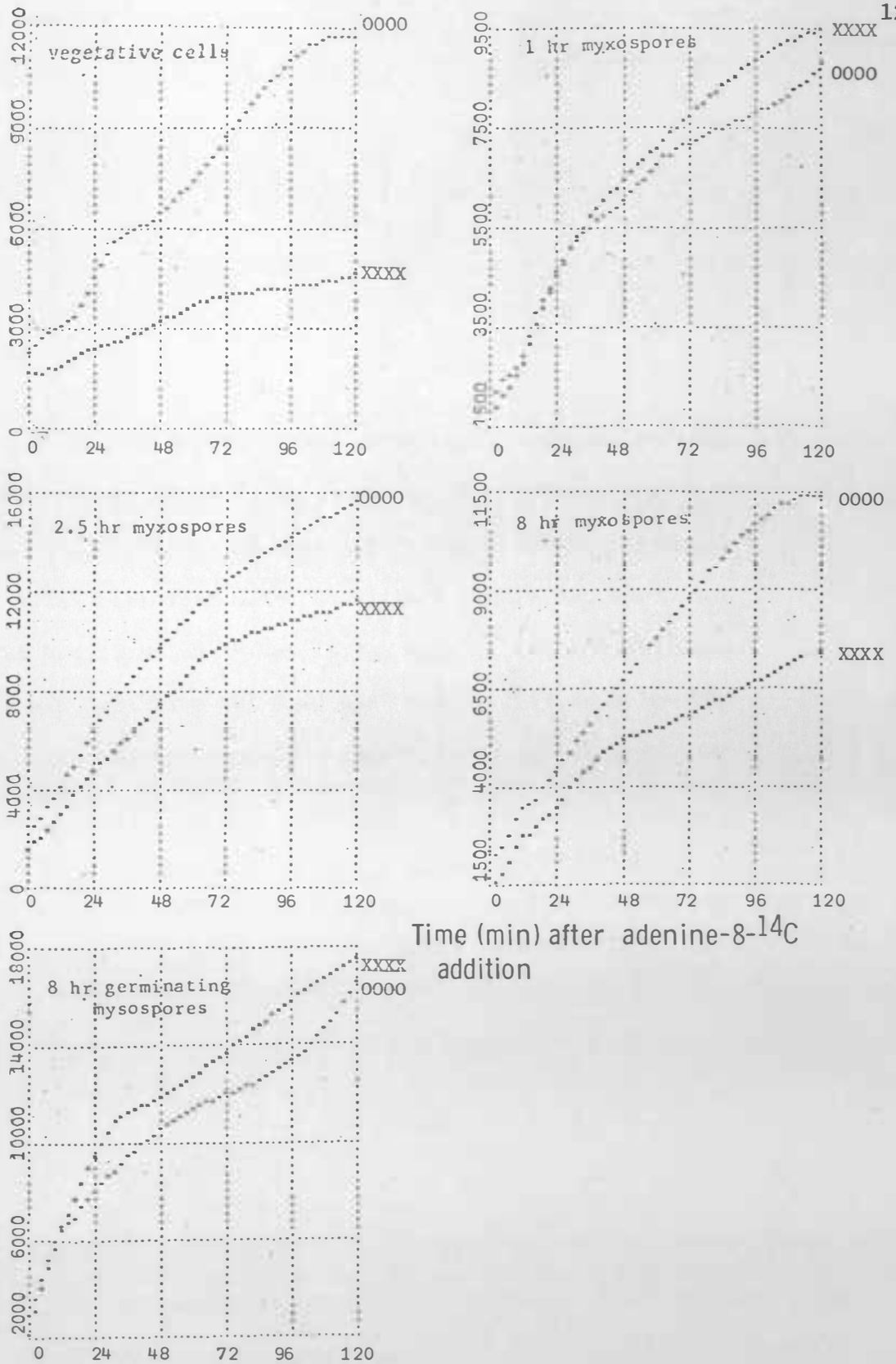
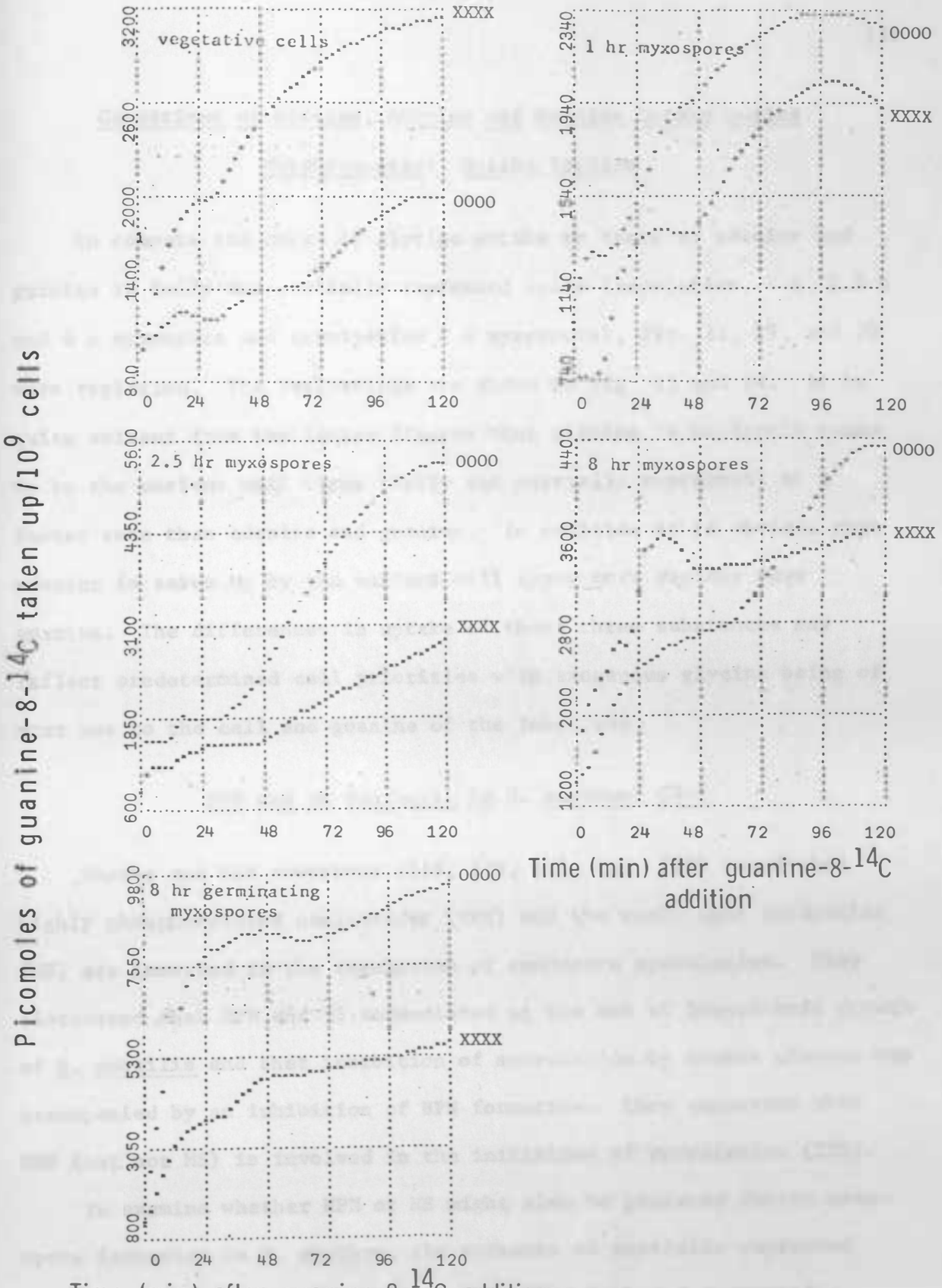

vegetative cells
0000
XXXX
0 3000 6000 9000 12000
0 24 48 72 96 120
1 hr myxospores
XXXX
0000
1500 3500 5500 7500 9500
0 24 48 72 96 120
2.5 hr myxospores
0000
XXXX
0 4000 8000 12000 16000
0 24 48 72 96 120
8 hr myxospores
0000
XXXX
1500 4000 6500 9000 11500
0 24 48 72 96 120
8 hr germinating mysospores
XXXX
0000
2000 6000 10000 14000 18000
0 24 48 72 96 120
Picomoles of adenine-8-14C taken up/10^9 cells
Time (min) after adenine-8-14C addition
Time (min) after adenine-8-14C addition

Fig. 22. Guanine uptake by vegetative cells, myxospores (1, 2.5 and 8 h) and germinating myxospores (8 h) of M. xanthus CW-1. Symbols: (OOOO), partially repressed cells; (XXXX), fully repressed cells. Partially and fully repressed cells were obtained and the guanine uptake assay was performed as indicated in Fig. 20 and MATERIALS AND METHODS. The graphs were rendered by a programmed minicomputer-teletype.


vegetative cells
XXXX
0000
3200
2600
2000
1400
800
1 hr myxospores
2340
1940
1540
1140
740
2.5 hr myxospores
5600
4350
3100
1850
600
8 hr myxospores
4400
3600
2800
2000
1200
8 hr germinating myxospores
9800
7550
5300
3050
800
0 24 48 72 96 120
Picomoles of guanine-8-14C taken up/10^9 cells
Time (min) after guanine-8-14C addition

Comparison of Glycine, Adenine and Guanine Uptake During Morphogenesis: Uptake Studies

To compare the rates of glycine uptake to those of adenine and guanine in fully and partially repressed cells (vegetative, 1 h, 2.5 h and 8 h myxospore and germinating 8 h myxospore), Fig. 12, 19, and 20 were replotted. The replottings are shown in Fig. 23 and 24. It is quite evident from the latter figures that glycine is uniformly taken up by the various cell types (fully and partially repressed) at a faster rate than adenine and guanine. In addition it is obvious that adenine is taken up by the various cell types more rapidly than guanine. The differences in uptake of these three substances may reflect predetermined cell priorities with exogenous glycine being of most use to the cell and guanine of the least use.

HPN and MS Synthesis in M. xanthus, CW-1

Rhaese and his coworkers (118, 119, 120, 121, 122) found that highly phosphorylated nucleotides (HPN) and the magic spot nucleotide (MS) are involved in the regulation of endospore sporulation. They discovered that HPN and MS accumulated at the end of logarithmic growth of B. subtilis and that inhibition of sporulation by excess glucose was accompanied by an inhibition of HPN formation. They suggested that HNP (but not MS) is involved in the initiation of sporulation (120).

To examine whether HPN or MS might also be produced during myxospore formation in M. xanthus, the extracts of partially repressed vegetative cells, myxospores (1 h, 2.5 h and 8 h) and germinating

Fig. 23. Glycine, adenine and guanine uptake by partially repressed vegetative cells, myxospores (1 h, 2.5 h and 8 h) and germinating myxospores (8 h) of M. xanthus CW-1. Symbols: (GGGG), glycine uptake; (AAAA), adenine uptake; (gggg), guanine uptake. Partially repressed cells were obtained and the glycine, adenine and guanine uptake assays were performed as indicated in Fig. 12, 19 and 20. The graphs were rendered by a programmed minicomputer-teletype.

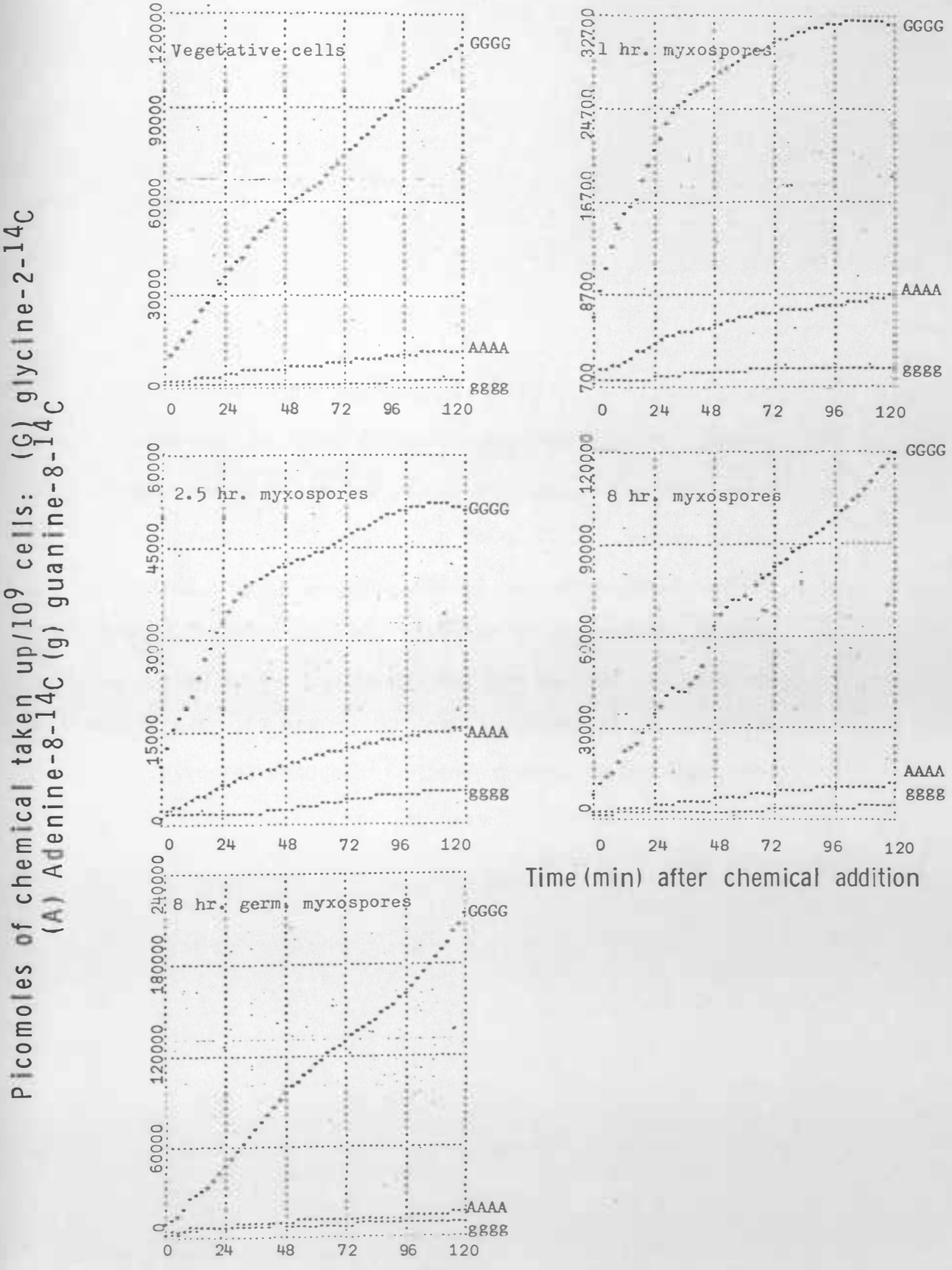
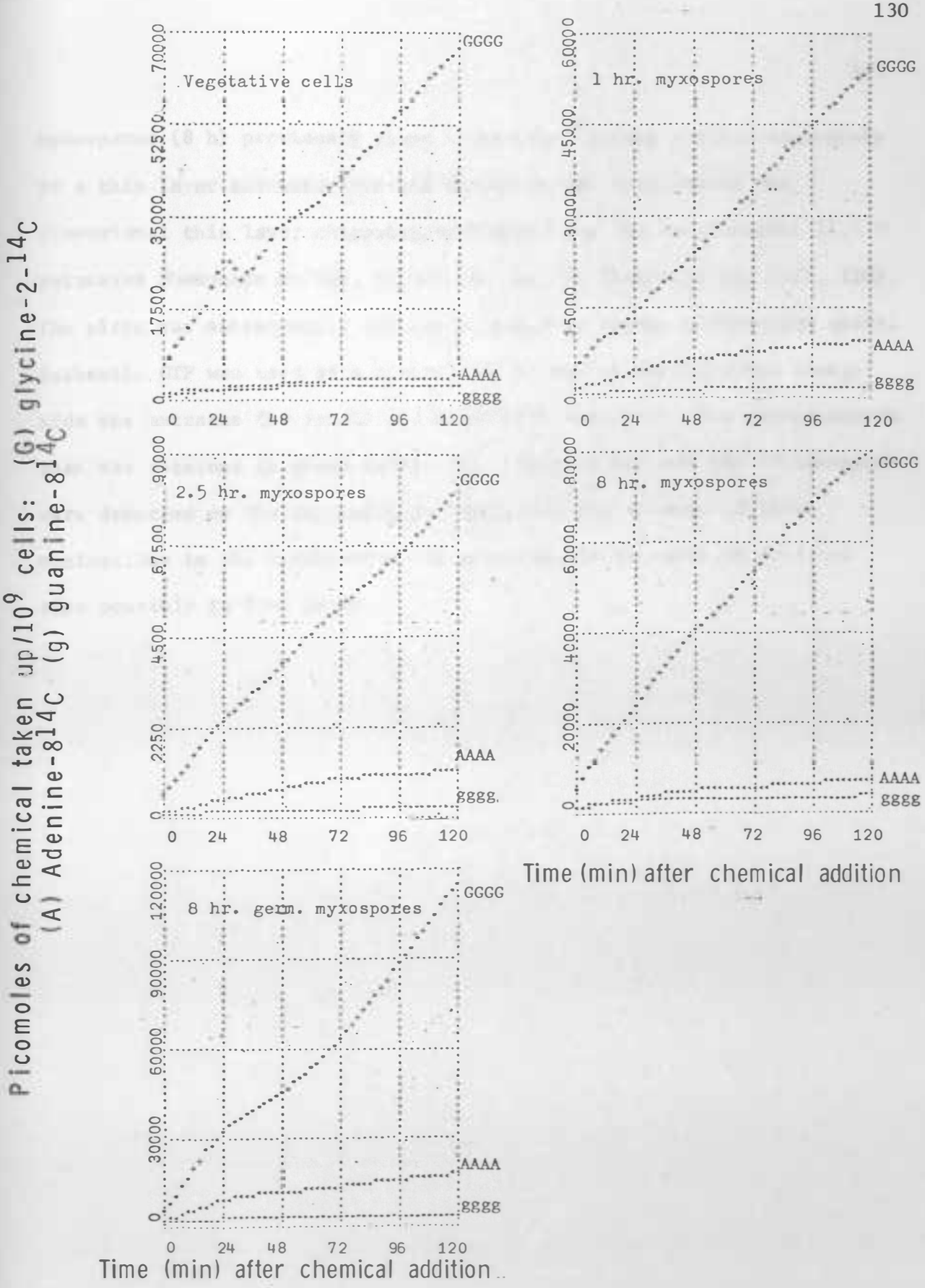

Picomoles of chemical taken up/10⁹ cells: (G) glycine-2-14C
(A) Adenine-8-14C (g) guanine-8-14C
Vegetative cells
GGGG
AAAA
gggg
1 hr. myxospores
2.5 hr. myxospores
8 hr. myxospores
8 hr. germ. myxospores
Time (min) after chemical addition
Time (min) after chemical addition

Fig. 24. Glycine, adenine and guanine uptake by fully repressed vegetative cells, myxospores (1, 2.5 and 8 h) and germinating myxospores (8 h) of M. xanthus CW-1. Symbols: (GGGG), glycine uptake; (AAAA), adenine uptake; (gggg), guanine uptake. Fully repressed cells were obtained and the glycine, adenine and guanine uptake assays were performed as indicated in Fig. 12, 19 and 20. The graphs were rendered by a programmed minicomputer-teletype.


Picomoles of chemical taken up/10^9 cells: (G) glycine-2-^{14}C
(A) Adenine-8^{14}C (g) guanine-8^{14}C
Vegetative cells
1 hr. myxospores
2.5 hr. myxospores
8 hr. myxospores
8 hr. germ. myxospores
GGGG
AAAA
gggg
0
24
48
72
96
120
Time (min) after chemical addition

myxospores (8 h) previously given glycine-2-^{14}C were applied separately to a thin layer chromatoplate and the plate was analyzed by one dimensional thin layer chromatography employing the same solvent (1.5 M potassium phosphate buffer, pH 3.4) as used by Rhaese et al. (119, 120). The plate was subsequently radioautographed to expose radioactive spots. Authentic GTP was used as a control and it was chromatographed alongside the extracts (by itself and mixed with extract). The radioautogram that was obtained is shown in Fig. 25. Neither HPN nor the MS compounds were detected on the chromatogram suggesting the absence of these nucleotides in the trichloroacetic acid-soluble extracts of CW-1 and also possibly in live cells.

Fig. 25. One-dimensional, thin-layer chromatographic-radioautographic analysis of M. xanthus CW-1 vegetative, myxospore and germinating cell extracts and GTP. Symbols: 1, GTP; 2, vegetative cell; 3, 1 h myxospore; 4, 2.5 h myxospore; 5, 8 h myxospore; 6, germinating 8 h myxospores; 7, 1 h myxospore extract + GTP. Abbreviations: A, UV absorbing spot. Partially repressed myxospores, vegetative cells and germinating cells were given glycine-2-^{14}C for 50-90 min and then were extracted according to the procedure in MATERIALS AND METHODS. Samples of 10 µl-30 µl of each cell extract and 5 mM GTP were applied separately to the bottom of a thin layer plate and the plate was chromatographed in one dimension (1.5 M potassium phosphate buffer, pH 3.4) and UV-radioautographically analyzed as described in MATERIALS AND METHODS.

GTP
A
A
A
A
A
GTP
1
2
3
4
5
6
7

DISCUSSION

Purine Nucleotide De Novo Synthesis in M. xanthus: In Vitro Studies

Synthesis during vegetative growth

The first indication of de novo purine nucleotide synthesis in M. xanthus came from the nutritional studies of Dworkin (21), who devised a completely synthetic medium lacking any purine that fully supported the vegetative growth of strain VC. The later discovery of purine-requiring mutants of strain FBa (prototroph) by Hemphill and Zahler (51) confirmed Dworkin's earlier findings.

My discovery of PP-ribose-P amidotransferase and ribose-5-P amino-transferase in vegetative cells of CW-2 M. xanthus likewise indicates the presence of a de novo purine route in this myxobacterium. My findings, in addition, suggest that the de novo route occurs in the same way that has been found to occur in other organisms (40, 130, 163).

The roles of PP-ribose-P amidotransferase and GAR synthetase in purine de novo synthesis have been well studied in many organisms (35, 40, 57, 90, 130, 162, 163). All evidence indicates that PP-ribose-P amidotransferase catalyzes the first step (reaction 1, Fig. 5) unique to de novo synthesis, and that GAR synthetase catalyzes the second (reaction 2, Fig. 5). The results from the substrate studies (Table 3, a, b, c) indicate this is also the case in M. xanthus. The alternative use of either L-glutamine or NH_4Cl as adequate nitrogen donors for

PP-ribose-P in the amidotransferase reaction has also been noted with the chicken liver enzyme (163), the Ehrlich ascites cell enzyme (147), the Friend-leukemia mouse spleen enzyme (115), the Burkitt lymphoma, and the human spleen enzyme (114). With the E. coli enzyme (75), NH_4Cl is a somewhat less than completely adequate replacement for L-glutamine, and with the A. aerogenes enzyme (98), it is totally inadequate. Hartman (48) has evidence that these two nitrogen donors bind to different substrate sites on the amidotransferase molecule. This could explain why the same enzyme from different organisms might respond differently to the two substrates.

Only one substrate site seems to exist per monomer of the amidotransferase for PP-ribose-P (163), and ribose-5-phosphate has not been found to replace PP-ribose-P in any form of the enzyme so far tested (47, 163).

Synthesis in the myxospore

My findings indicate that PP-ribose-P amidotransferase is present in the myxospore as well as the vegetative cell. This is interesting in view of the results of Switzer and coworkers (144, 149, 150) on B. subtilis who observed an inactivation of the amidotransferase during endospore formation.

The scope of the amido- and aminotransferase assay results is unfortunately too limited to draw any unequivocal conclusions concerning in vivo purine synthesis in the intact myxospore. The possibility still existed despite the results of this part of my study that the enzymes,

while present and measurable in myxospore extracts, did not actually function *in vivo* in the whole cell because of stringent regulatory controls (inadvertently removed during myxospore extraction). Two such controls seemed possible, feed-back inhibition of the first *de novo* enzyme imposed by high intracellular levels of nucleotides and dormancy imposed shut-down of one or more of the enzymes (possibly through substrate limitation or dehydration).

My enzyme assay findings when viewed against the results of Hanson and Dworkin (45) seem to rule out feed-back inhibition as a major deterring force of myxospore *de novo* synthesis. AMP, GMP, ADP, GDP and GTP are the most potent inhibitors of the amidotransferase (Table 4) but the inhibition imposed by the two most powerful of these inhibitors, AMP and GMP (and presumably the other nucleotides), drops off sharply as the concentration is reduced from 6.25 mM to 1-2 mM (Fig. 9) and is insignificant at the lower level. The lower concentration, 1-2 mM, is noteworthy because from the work alluded to above of Hanson and Dworkin, it would appear [from calculations and assumed myxospore volume of 4 μ^3, (24)] to correspond very nearly to the additive intracellular concentration in the myxospore of all of the above important amidotransferase inhibitors. Unless the intracellular nucleotides impose an extravagantly synergistic inhibitory effect on the amidotransferase, it seems inescapable that major feed-back inhibition of *de novo* purine synthesis is missing in the intact myxospore.

Ribose-5-phosphate aminotransferase

Although the roles of PP-ribose-P amidotransferase and GAR synthetase in de novo purine synthesis are clear, the role of ribose-5-phosphate aminotransferase (reaction 1a) in this process remains clouded. Reem (113) purified (almost 100-fold) an enzyme from chicken liver that demonstrated ribose-5-phosphate aminotransferase activity. Le Gal et al. (75) provided evidence for such an enzyme in E. coli, as did Kapoor and Waygood (64) in wheat, Herscovics and Johnstone (55) in Ehrlich ascites cells, and Reem (113) in Burkitt lymphoma and human spleen tissue. But Nierlich and Magasanik (99), on the other hand, demonstrated that the formation of PRA from ribose-5-phosphate and NH_4Cl could occur non-enzymatically under certain conditions, and Gots (40) and Wyngaarden (163) have questioned the significance of the aminotransferase in de novo synthesis on the basis of the properties of pur-F mutants of S. typhimurium (35). These mutants have a strict growth requirement for added purine and lack the amidotransferase but yet demonstrate ribose-5-phosphate aminotransferase activity. Gots (40) has suggested that the intracellular ammonium concentration may be too low, or that a rapid conversion of ribose-5-phosphate to PP-ribose-P may not permit sufficient substrate supply to allow the aminotransferase to function in vivo. In hepatoma cells, indirect evidence implies that ribose-5-phosphate aminotransferase may function in the purine de novo synthesis of this cell line (85). Evidence, albeit not entirely conclusive, favoring the existence of an enzyme with aminotransferase activity in M. xanthus comes from the -20 C storage stability and feed-back inhibition results.

Assuming little or no feed-back inhibition of the assay la coupling enzyme or of non-enzymatic PRA synthesis, my findings suggest that AMP and cyclic AMP are the only significant physiological inhibitors of the ribose-5-phosphate aminotransferase of M. xanthus.

The inhibition of ribose-5-phosphate aminotransferase by cyclic AMP observed in vitro could have been just coincidental, due to the closeness in structure between cyclic AMP and AMP. AMP may serve as the true inhibitor in vitro, whereas cyclic AMP has no real intracellular inhibitory function. Another possibility, considering the crude extract system, is that cyclic AMP is converted to AMP by cyclic AMP phosphodiesterase (108) and that what was observed in the tests was an inhibition by secondarily generated AMP. This secondary effect might also explain the observed inhibition of PP-ribose-P amidotransferase by cyclic AMP, for other investigators (Gots, personal communication, 57) have observed no significant cyclic AMP inhibition of purified S. typhimurium and human placental PP-ribose-P aminotransferase.

Since crude extracts were used in my de novo studies, interference from enzymes such as the phosphodiesterase discussed above, nucleoside di- and triphosphate phosphodiesterases (removing pyro- or orthophosphate from the nucleoside di- and triphosphates used as inhibitors), and other enzymes cannot be entirely discounted. For example, PP-ribose-P synthetase (EC 2.7.6.1), if present and active in the crude extracts (130), theoretically could have converted ribose-5-phosphate (and ATP) to PP-ribose-P (and AMP) in assay la mixtures, thereby interfering with

the aminotransferase reaction. Although this is hypothetically possible, actual interference by the synthetase under the conditions of our system seems at best minimal because (i) GMP and certain other purines that strongly inhibited (i.e. 100% in the case of GMP at 6.25 mM) the amidotransferase-dependent reaction only weakly inhibited the aminotransferase-dependent reaction (i.e. 16.5% in the case of GMP at 12.5 mM); and (ii) if the synthetase were significantly interfering, then (e) and (h) of Table 3 should have approximated one another or been identical just as (a) and (b) were identical, since L-glutamine and NH_4Cl serve as equally good nitrogen donors for the amidotransferase.

A more likely interfering enzyme in the crude extract system may be purine nucleoside phosphorylase (40). The marked difference in guanosine inhibition of the amido- versus the aminotransferase (Table 4) might then be explained by the phosphorylase-mediated conversion of guanosine to guanine and ribose-1-phosphate, the concomitant transformation of the latter to ribose-5-phosphate and a stimulatory substract effect of the 5'-sugar on the aminotransferase (but not the amidotransferase) reaction. On the other hand, Vahora (153) has observed that the aminotransferase of S. typhimurium is inhibited rather than stimulated by concentrations higher than 10 to 15 mM, the level used in assay 1a. A similar inhibition of other ribose-5-phosphate aminotransferases by such levels of ribose-5-phosphate has also been noted by Reem (113, 114).

PP-ribose-P amidotransferase

With M. xanthus CW-2 (vegetative cell and myxospore) extracts, GAR production from ribose-5-phosphate and NH_4Cl (via the aminotransferase) was always less than from PP-ribose-P and L-glutamine (via the amidotransferase). This has also been noted by Reem (113, 114) with the chicken liver and human spleen enzymes. Reem (114), on the other hand, noted with the Burkitt lymphoma form of the enzyme a somewhat higher capability to produce GAR from ribose-5-phosphate and NH_4Cl than from PP-ribose-P and L-glutamine. Le Gal et al. (75) observed about equal capabilities with the E. coli enzyme.

AMP, ADP, GMP, and GDP were the most effective inhibitors of the PP-ribose-P amidotransferase of M. xanthus CW-2, whereas adenosine, guanosine, IMP, GTP, and 3':5' cyclic AMP were less effective, and UMP, CMP, CTP, and ATP were ineffective or only slightly effective. This inhibition pattern evidences certain similarities with what has been observed in other bacteria. The S. typhimurium enzyme (40), for example, is also strongly inhibited by both AMP and GMP, as is the A. aerogenes enzyme (98), whereas the B. subtilis enzyme (136) is most strongly inhibited by AMP and ADP. GDP was either not tested on these other bacterial enzymes or it had only a small effect. In A. aerogenes and B. subtilis, ATP and the pyrimidine nucleotides tested were ineffective inhibitors of the amidotransferase. In other systems where the amidotransferase has been examined for feed-back inhibition, different combinations of purine nucleotides have been found to be inhibitory, with AMP and GMP being the most commonly effective combination (40, 57, 163).

The existence of a separate binding site(s) for AMP and GMP on the amidotransferase of M. xanthus apart from the catalytic site is suggested by the 2.2 n' value that was obtained for both nucleotides. Other PP-ribose-P amidotransferases also possess n' values of around 2 (163), indicating the allosteric nature of these and probably all PP-ribose-P amidotransferases.

Purine-Thiamine De Novo Synthesis in M. xanthus: In Vivo Studies

Glycine uptake

The in vitro enzyme assays employed in the initial stages of my study successfully detected certain purine-thiamine de novo enzymes in vegetative and myxospore cells of M. xanthus. The discovery of these enzymes in these two important cell forms and the enzymes feed-back inhibition behavior suggests that purine de novo synthesis can function throughout the life cycle of this microorganism. This suggestion is affirmed by the glycine uptake and TLC-radioautographic studies.

The glycine uptake study was premised on the earlier works of White (personal communication) and Ramsey and Dworkin (110), who had previously demonstrated the ability of various cell stages of M. xanthus to take up glycine. My results confirm their findings but additionally my results indicate something not known before, that partially repressed cells (of all types) take up glycine more rapidly than fully repressed cells. This suggests that purine de novo synthesis occurs continuously throughout the life cycle of partially repressed M.

xanthus cells. An apparently different situation exists in another spore-forming bacterium, B. megaterium. Setlow and Kornberg (135) found that the endospore of this organism is incapable of incorporating exogenous glycine into purine nucleotides but that the vegetative cell does possess this ability. This suggests that the permeability of the endospore is different from that of the vegetative cell (unlike M. xanthus) or that a regulatory device present in the endospore but absent in the vegetative cell conserves exogenously provided precursors such as glycine.

Fully repressed M. xanthus cells, assumed to be devoid of purine de novo synthesis, still take up significant amounts of glycine. This can be accounted for in terms of: (i) myxospore coat formation (72); (ii) protein synthesis (55); (iii) glyoxylate and α-amino acid formation (53); and (iv) gluconeogenesis in the myxospore (156).

The increased uptake of glycine by partially repressed vegetative, myxospore and germinating cells suggests that purine de novo synthesis occurs throughout the life cycle of M. xanthus. The increased glycine uptake may not necessarily be due to purine de novo synthesis, however, but could alternatively be due to usage of glycine in another pathway that also uses glycine as a precursor. This could have come about if dialysis of the casitone, used for growth and maintenance, removed metabolites (e.g. amino acids) whose synthesis by M. xanthus required glycine. Their removal from the casitone would have derepressed the corresponding synthetic pathways in M. xanthus cells (assuming them to

be present) grown or maintained in dialyzed casitone medium and consequently there would have occurred an increased uptake of glycine due to its usage in a pathway (or pathways) other than purine de novo synthesis. Furthermore, if non-dialyzable polynucleotides or nucleic acids were present in the dialyzed casitone they would most certainly have been hydrolyzed by the active nucleases of M. xanthus (51) producing exogenous purines (or derivatives) and preventing the stated purpose of the dialysis, partial derepression of the purine de novo pathway. Thus a condition may have developed in dialyzed casitone cultures where M. xanthus cells became completely repressed of the purine de novo pathway and completely derepressed of other glycine utilizing pathways. Increased glycine uptake would have then meant not purine de novo synthesis but glycine usage in some other pathway. This alternative explanation for increased glycine usage although theoretically possible seems not to be too likely because: (i) M. xanthus cells possess active proteases (104), which would have hydrolyzed non-dialyzable peptides and proteins in the dialyzed casitone to amino acids preventing derepression of any of the amino acid synthetic pathways; and (ii) there is no evidence that casitone contains significant levels of non-dialyzable polynucleotides or nucleic acids.

An interesting pattern emerges if the rate of glycine uptake during myxospore induction is compared to the rates of nucleic acid synthesis that occur during the same time. The rate of glycine uptake drops off by about 50% (compared to vegetative state) during the first hour of glycerol induction while DNA synthesis continues at an abated rate

(123), net RNA synthesis stops and RNA turnover commences (127). These events indicate that perhaps during induction, no or little glycine is committed to making new RNA purine nucleotides, a lesser amount is used for DNA synthesis and that the savings is translated into lessened glycine uptake by the maturing myxospore. (Possible mechanism for reducing usage of glycine in purine nucleotide pathway is discussed on page 154.)

The increase in the rate of glycine uptake that occurs during glycerol-induced myxospore germination may reflect the necessity for an increased metabolic rate of the germinating cell. In keeping with this hypothesis is the report of Dworkin (personal communication) that germinating myxospores have a faster growth rate than vegetative cells.

Glycine conversion to purine nucleotides

The production of radioactive ATP and GTP from glycine-2-^{14}C by vegetative cells, myxospores and germinating cells suggests, as do the other results, that purine de novo synthesis can occur throughout the life cycle of M. xanthus. Caution must be taken in interpreting the TLC-radioautographic results, however, because they rest upon the assumption that carbon 2 of glycine only becomes incorporated into purine nucleotides by the de novo route and this assumption may or may not be strictly true. Henderson (53) has clearly enunciated the cautionary stance that has to be taken in using radioactive glycine to study de novo synthesis, "care must be taken in studies of labeling of purines by glycine to be sure that the label does in fact represent

incorporation of the whole glycine molecule and not just randomization of fragments derived from other routes of glycine metabolism." Other routes of glycine metabolism (apart from purine de novo synthesis) which theoretically could have led to the incorporation of carbon 2 of glycine into the purine nucleotides of M. xanthus are indicated in Fig. 26.

The secondary routes of glycine usage in ATP and GTP synthesis, outlined in Fig. 26, involve the deployment of glycine carbon into the ribose moiety of ATP and GTP either by way of de novo synthesis or by way of salvage synthesis. The occurrence of either of these steps and the usage of glycine at the PRA step of de novo synthesis would lead to ATP and GTP molecules containing glycine carbon in both the purine ring and the ribose moiety. One of the secondary routes of glycine usage in ATP and GTP synthesis outlined in Fig. 26 involves glyoxylate. It is conceivable that glycine conversion to glyoxylate could occur in M. xanthus by way of either glutamate-glyoxylate aminotransferase or alanine-glyoxylate aminotransferase, both of which have been found in vegetative cells and myxospores (73). The glyoxylate so formed could subsequently be channeled into the TCA cycle by way of malate synthase which converts glyoxylated to malate and which increases significantly in level during glycerol induced myxospore formation (accompanied by parallel increase in isocitrate lyase activity) (103). Subsequent usage of a gluconeogenic pathway and transketolase rearrangement would generate ribose-5-phosphate which could be used in purine salvage synthesis. The availability of these enzymes and pathways does not

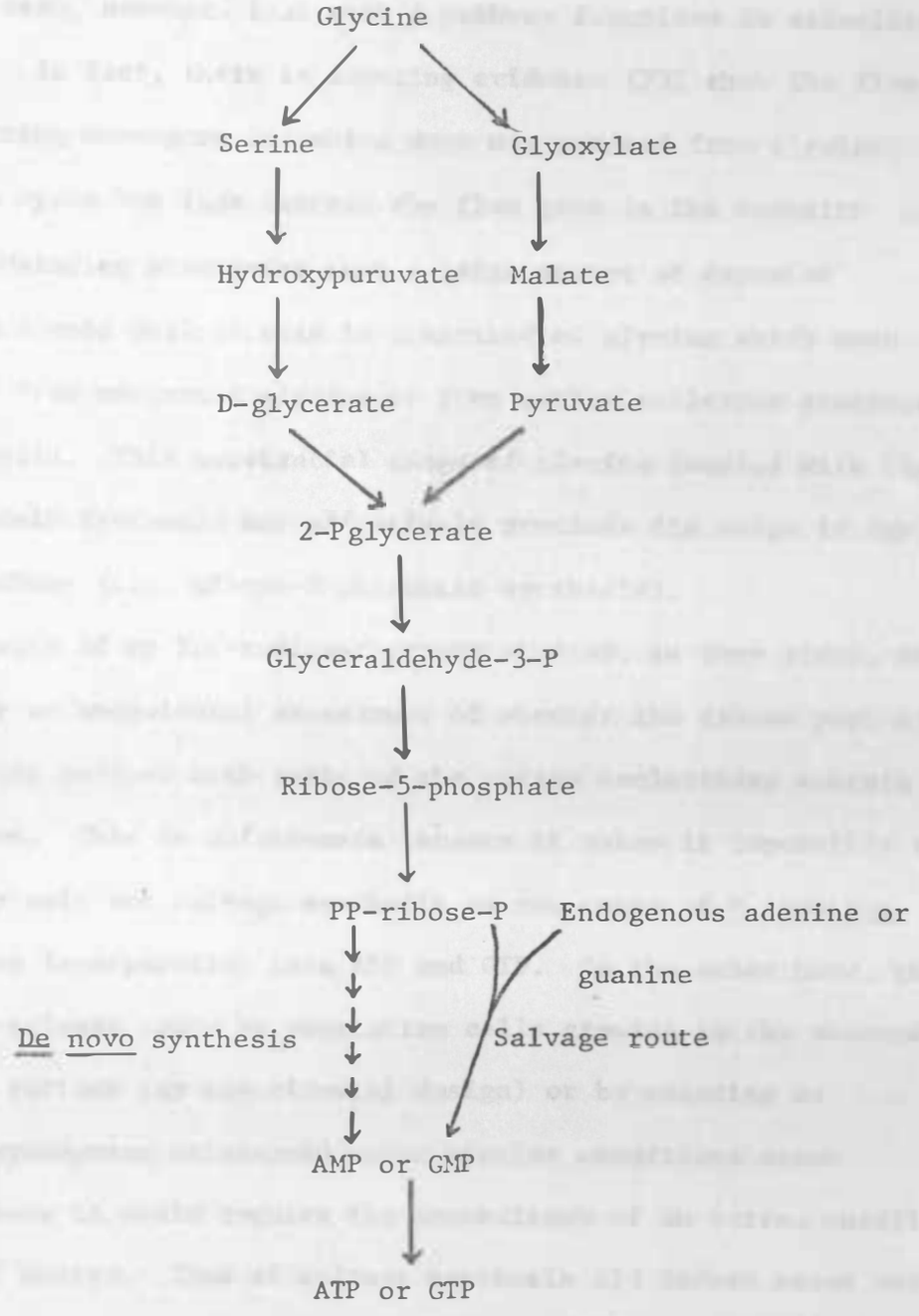


Fig. 26. Possible secondary routes of glycine usage in ATP or GTP synthesis.

necessarily mean, however, that such a pathway functions in actuality in this way. In fact, there is mounting evidence (73) that the flow of carbon during myxospore induction does not proceed from glycine into the TCA cycle but that instead the flow goes in the opposite direction. Maturing myxospores form a large amount of capsular material and a good deal of this is comprised of glycine which must arise either from exogenous glycine or from carbon skeletons generated in the TCA cycle. This substantial usage of glycine coupled with its usage in protein synthesis may effectively preclude its usage in any secondary pathway (i.e. ribose-5-phosphate synthesis).

The results of my TLC-radioautography studies, as they stand, do not allow for an unequivocal assessment of whether the ribose part or the purine ring part or both parts of the purine nucleotides contain glycine carbon. This is unfortunate because it makes it impossible to unequivocally rule out salvage synthesis as the cause of M. xanthus glycine carbon incorporation into ATP and GTP. On the other hand, the usage of the salvage route by vegetative cells growing in the absence of exogenous purines (my experimental design) or by maturing or germinating myxospores maintained under similar conditions seems unlikely because it would require the expenditure of an extra, uncalled for amount of energy. Thus if salvage synthesis did indeed occur under these conditions, it would require that AMP and GMP be dephosphorylated or dephosphoribosylated and that the nucleosides or free bases formed as a consequence be rephosphorylated or rephosphoribosylated (see Fig. 8

in REVIEW OF LITERATURE) and this would have required extra energy via ATP utilization.

To resolve the uncertainty arising from the TLC-radioautography results, our laboratory is presently examining the radioactive ATP and GTP observed on the TL chromatograms to determine whether the ^{14}C labelling occurs in the ribose or purine ring parts of the nucleotides.

Another uncertainty about interpreting the TLC-radioautography results concerns the extent of UTP contamination of ATP on the chromatograms. If the substance thought to be ATP on the chromatograms was actually UTP or ATP heavily contaminated with UTP, then the conclusion that ATP is formed de novo by M. xanthus would be invalid or at least premature, because that conclusion requires that the chromatogram substance be comprised in part or total of ^{14}C ATP (and synthesized from glycine-2-^{14}C). On the other hand, it would appear likely that most of the material on the chromatogram spots thought to be ATP, actually is ATP because M. xanthus cells (and supposedly extracts prepared from such cells) contain from 2.5 to 6 times as much ATP as UTP (45) and no other spots on the chromatograms resembled ATP. Furthermore, any contaminating UTP would probably have contained low radioactivity since glycine is only an indirect precursor of UTP, contributing carbon to this nucleotide via aspartate (53) or the routes described in Fig. 26.

Function of Purine De Novo Synthesis in the Myxospore

The presence of a functional de novo synthetic pathway in the myxospore of M. xanthus should result in the continuous production of purine nucleotides unless the proper substrates are unavailable. Most of the purine nucleotides synthesized during induction would most likely end up in new DNA or in the adenylate pool and little would apparently be used for new RNA synthesis (see page 143). Continued DNA synthesis appears to be necessary to complete a final round of chromosome replication (168) and maintaining a proper size adenylate pool may be necessary for myxospore completion and germination (e.g. ATP is utilized indirectly to produce GTP for protein synthesis, UTP for cell wall synthesis and CTP for membrane synthesis).

The presence of a functional purine de novo pathway in the so-called "dormant" myxospore is somewhat surprising in light of the absence of this pathway in the endospore. This may reflect major differences between the myxospore and endospore which indeed is manifested in other differences between the two types of spores (e.g. adenylate pool size).

Purine Interconversion and Salvage in M. xanthus

The first indication that purine interconversion and salvage are present in M. xanthus came from the finding that purine bases, nucleosides, or nucleotides can support the growth of purine-requiring mutants (51). My finding that both adenine and guanine can be taken

up by vegetative cells, myxospores and germinating cells presents direct evidence for the existence of purine interconversion and salvage in all cell forms of M. xanthus. Because there occurs no lag period for either adenine or guanine cellular incorporation, it seems likely that the enzymes responsible for their transport into cells, adenine and guanine phosphoribosyltransferase, are constitutive rather than inducible.

Berlin and Stadtman (5), studied the uptake of radioactive adenine and guanine by resting cells of B. subtilis and found that the incorporation of both proceeds in step-like increments. My results indicate that guanine may also be taken up into vegetative cells and myxospores (1 h, 2.5 h and 8 h) in step-like increments (see Fig. 22), however, adenine seems to be taken up into those cell types in a relatively linear fashion (see Fig. 21). The step-like uptake of guanine may indicate a finely tuned control of guanine phosphoribosyltransferase possibly mediated through a nonlinear feed-back inhibition of the enzyme by increased intracellular levels of guanine nucleotides (GMP or GTP).

In B. subtilis, the adenine phosphoribosyltransferase has been found to be associated with membrane vesicles, and adenine uptake by membrane preparations is completely correlated with enzyme activity (5). In M. xanthus, similar vesicular structures are localized between the coat and cell wall of glycerol-induced myxospores (2) and it has been suggested that these vesicles may play some role in germination (137). The rapid uptake of purines by germinating cells that I observed

may hence be correlated with the functioning of such vesicles in germination. This suggests the need for a plentiful supply of purines (or purine nucleotides) during germination.

From the results of my study, it is hard to evaluate the role of purine interconversion and salvage in morphogenesis. The tendency for the level of purine nucleotide pools to increase during glycerol-induced myxospore formation (45) may be accounted for, in part, by purine interconversion and salvage.

HPN and MS Synthesis in M. xanthus, CW-1

Highly phosphorylated nucleotides (HPN) were originally found in sporulating cells of B. subtilis (118, 120, 121, 122) and in differentiating mammalian cells (117) such as Chinese hamster ovary cells, baby hamster kidney cells and human lung cells. These studies prompted the suggestion that the HNP are involved in regulation of morphogenesis in a wide variety of biological systems (120). The magic spot (MS), another type of unusual nucleotide, usually accumulates in E. coli (16) and B. subtilis (119) after starvation for a required amino acid. It is believed that MS is involved in control of ribosomal RNA synthesis (16). In my studies of M. xanthus, neither HPN nor MS were detected in various types of glycerol-induced myxospores (1 h, 2.5 h and 8 h).

These nucleotides may be truly missing in M. xanthus or alternatively my extraction procedure, which was different from the standard method (118, 120), may have missed picking up these nucleotides and this may account for their apparent absence. On the other hand, the

technique I used (trichloroacetic acid extraction) detected other purine nucleotides and it seems unlikely that it would have been so exclusive as to have missed HPN and MS.

Possible Involvement of Thiamine in Myxospore Formation

Schaeffer et al. (133) in 1965 proposed a theory of catabolic repression of bacterial sporulation which suggests that an intracellular nitrogen-containing metabolite controls, directly or indirectly, the expression of all sporulation genes. Evidence (46, 131) which has accumulated since then supports this theory and indicates that the release of catabolite repression is the earliest relevant regulatory event causally connected to sporulation. Since the sporulation of various bacilli can be made to occur when growth is limited by glucose (133), nitrogen (42, 133), or phosphate (42, 82), the catabolite repressor has been hypothesized to be an effector containing carbon, nitrogen and phosphate (58).

What is the nature of the effector that controls bacterial morphogenesis? In E. coli, cyclic AMP has been implicated in catabolite repression (106), however, this compound has not been detected in M. xanthus (45) or bacilli (59, 134). HPN may be involved in bacilli sporulation (118, 119, 120, 121, 122), however, attempts (132) to correlate the appearance of HPN or MS with sporulation in B. subtilis have failed. My results with the TLC-radioautographic analysis of trichloroacetic acid extracts of M. xanthus did not reveal the presence of HPN or MS or any similar compounds.

A temporary decrease in the energy charge (the ATP pool levels) was proposed to be a regulatory signal for the early events of sporulation in bacilli (58). The finding of no energy charge change in M. xanthus before or during myxospore formation (45) seems to rule out this sort of regulatory signal as the cause of myxobacterial sporulation.

Elmerich and Aubert (32) found that a B. megaterium mutant unable to synthesize glutamine, a de novo precursor of purine-thiamine, sporulated well in a glutamine-deficient minimum medium but did not sporulate in media containing glutamine. They suggested from other evidence that the sporulation-repressive effect exerted by glutamine was caused by the purine de novo pathway, in that it either permitted the synthesis of a repressor (corepressor or catabolite repressor) or prevented the synthesis of a sporulation-inducing compound. To test their hypothesis, they isolated a purine-requiring mutant blocked in an early step of purine de novo synthesis (localized between PP-ribose-P and AIR, see Fig. 4) and they found that this mutant would sporulate in the presence of glucose and ammonia but that later blocked purine-requiring mutants (after AIR) and the wild type would not sporulate under these conditions. The fact that later blocked mutants were still effected by glucose-ammonia while early blocked mutants were not, suggests that the effector of sporulation: (i) is not a purine nucleotide; and (ii) is an early intermediate of de novo synthesis or a product produced by a pathway having common early steps with purine de novo synthesis. Since the thiamine synthetic pathway in bacteria

has common early steps with purine de novo synthesis (95), it is possible that thiamine is a sporulation effector or participates in the formation of a sporulation effector. The fact that it contains carbon nitrogen and phosphate (in form of thiamine pyrophosphate) is also in keeping with this function.

The thiamine-like substance observed on one- and two-dimensonal TLC in my studies may represent a sporulation effector in M. xanthus. Unfortunately, evidence for this speculation is somewhat meager and indirect. One piece of evidence is that the thiamine-like substance is produced in quantities larger than would be expected if it were simply acting as a coenzyme and nothing else. The second piece of evidence is that the substance seems to decrease and increase in quantity during myxospore formation in exactly the way that would be expected if it were the repressor. Thus it decreases in quantity during the early stages of induction but increases during the later stages of maturation (once myxospore genes have functioned they should be turned off in preparation for germination).

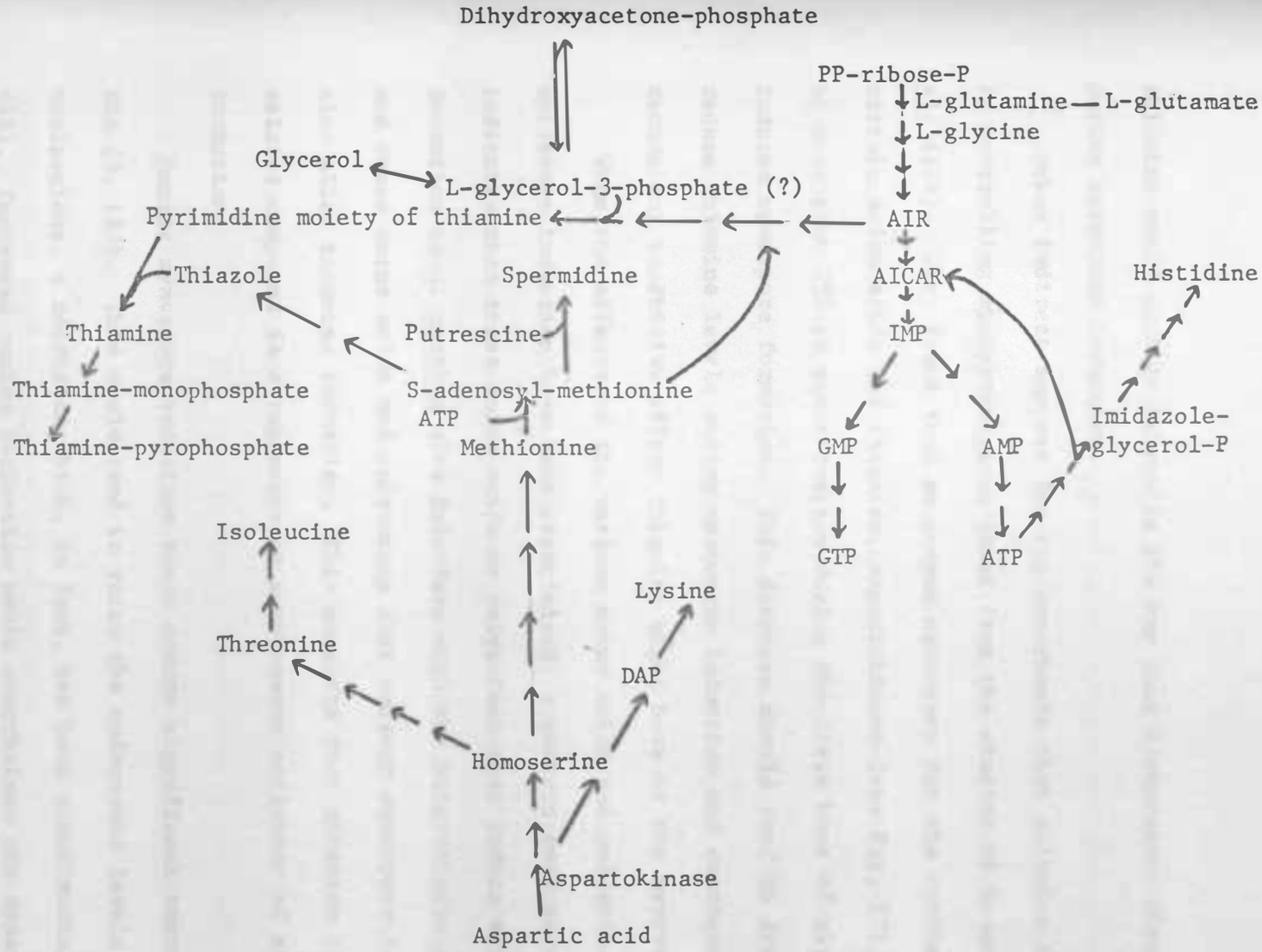
Nutritional studies indicate that M. xanthus can grow in a thiamine-lacking, chemically-defined medium (21, 51, 160). This suggests that thiamine can be synthesized de novo in this organism. External thiamine at vitamin levels has no stimulatory or inhibitory effect on the growth of M. xanthus (21) and may not enter the cell.

Myxospore formation in M. xanthus is subject to nutritional control (28, 30, 160). Witkin and Rosenberg (160) found that myxospore formation could be induced in a chemically defined medium by methionine

limitation or by the addition of feed-back inhibitors of methionine synthesis (threonine plus isoleucine). Spermidine, a product of further methionine metabolism, is also an inhibitor of myxospore formation. It is formed from the reaction of putrescine, a myxospore inducer, with decarboxylated S-adenosyl-methionine (89). S-adenosyl-methionine is a methylation product of methionine and ATP (145). All of the aforementioned compounds are related in some way or another to thiamine synthesis and these relationships as well as the role of purine de novo synthesis in thiamine formation are indicated in Fig. 27.

A 50% decrease in endogenous spermidine concentration occurs during the myxospore induction triggered by high concentrations of isoleucine-threonine (160). The decrease in spermidine is most likely preceded by a decrease in the concentration of S-adenosyl-methionine, a precursor of spermidine. Since methionine (possibly in the form of S-adenosyl-methionine) is a methyl-group donor for the thiazole portion of thiamine (60) and since it also serves as a cofactor in the biosynthesis of the pyrimidine moiety of thiamine (94), its decrease in vegetative cells would slow down the biosynthesis of thiamine. Thus, the amount of thiamine in M. xanthus should be proportional to the intracellular concentration of methionine, S-adenosyl-methionine and spermidine. Since spermidine is decreased in quantity during myxospore induction, it is reasonable to assume from the above considerations that thiamine would also be decreased in quantity during myxospore induction. This is important because the hypothetical repressor of spore genes should also decrease in quantity in this way and therefore

Fig. 27. Metabolic relationships between purine nucleotides, thiamine, histidine, glycerol and amino acids of aspartate family. The abbreviations used are the same as those used in Fig. 4.


Dihydroxyacetone-phosphate
PP-ribose-P
L-glutamine
L-glutamate
L-glycine
Glycerol
L-glycerol-3-phosphate (?)
Pyrimidine moiety of thiamine
AIR
Thiazole
Spermidine
AICAR
Histidine
Thiamine
Putrescine
IMP
Thiamine-monophosphate
S-adenosyl-methionine
ATP
Imidazole-glycerol-P
Thiamine-pyrophosphate
Methionine
GMP
AMP
Isoleucine
GTP
ATP
Lysine
Threonine
DAP
Homoserine
Aspartokinase
Aspartic acid

thiamine would seem to behave in the way that a repressor should behave during myxospore induction.

Other indirect support for the hypothesis that thiamine is involved in controlling myxospore genes comes from the studies of Rosenberg *et al.* (124). They found that an enzyme necessary for the synthesis of certain amino acids and thiamine, aspartokinase (see Fig. 27), decreases by more than 75% in concentration during the first hour of glycerol-induced myxospore formation. This decrease should tend to dramatically reduce thiamine levels during myxospore induction and consequently remove any repressive effect thiamine might have on the myxospore genes.

When the effects of the various amino acids and polyamines on myxospore induction have been scrutinized, a pattern emerges which indicates that those amino acids or polyamines that induce myxospore formation in *M. xanthus* also interfere with the biosynthesis of thiamine and those amino acids and polyamines that prevent myxospore formation also allow thiamine formation. This suggests that thiamine or a related compound is a repressor or a repressor activator of myxospore formation.

During myxospore induction there occurs significant turnover of RNA (3, 127). This would tend to raise the endogenous levels of purine nucleotides, a condition which, in fact, has been experimentally noted (45). Increased purine nucleotide pools nonetheless are apparently not sufficient to significantly repress the first steps of purine *de novo* synthesis, as discussed previously (page 135). The decreased uptake of glycine committed to *de novo* synthesis that occurs during

early myxospore induction (Table 5) thus is unexplained, if indeed purine synthesis does continue unabated during myxospore induction. A possible explanation is that B_1 pyrimidine formation (which uses early purine de novo steps) is discontinued during induction reducing the total amount of glycine used in the de novo pathway. This explanation is appealing because it would also dovetail nicely with the proposal that a thiamine-like substance is the repressor (catabolite or co-repressor) of myxospore induction. Such a repressor should be present during vegetative growth but should disappear during myxospore induction.

The renewed and elevated glycine uptake that occurs during the later stages of myxospore formation and germination may reflect glycine usage in myxospore and germination specific events (see earlier discussion) and also possibly for the renewed synthesis of B_1 pyrimidine. The latter would be expected if a B_1 pyrimidine product (i.e. thiamine-like substance) was indeed the repressor of these genes and if the activity of myxospore genes was only required for a short time span during induction. The turning off of these genes by the proposed thiamine-like repressor would then ready the organism for eventual germination.

Relationship between glycerol and thiamine in myxospore formation

Besides being influenced by amino acids and polyamines, sporulation in M. xanthus is also effected by glycerol. Dworkin and Gibson (28) were the first to observe that high concentrations of glycerol (0.5 M)

induce rapid and synchronous myxospore formation in M. xanthus. Sadler and Dworkin (127) were unable to ascertain the mechanism of glycerol induction but they speculated because they had observed so little glycerol uptake by the inducing cells that the vegetative cell membrane surface is the site of action. They suggested that it is effected in such a way by glycerol that specific DNA synthesis is inhibited and hitherto dormant myxospore genes are provoked into action.

It is possible that the inducing effect of glycerol is directly related to the similar effect of the aforementioned amino acids and is mediated through thiamine. The link may be via L-glycerol-3-phosphate. This metabolite is a commonly occurring intermediate in a variety of spore forming bacteria (21, 34). In B. subtilis it is normally converted to dihydroxyacetone-phosphate by way of glycerolphosphate dehydrogenase and in mutants devoid of this enzyme, L-glycerol-3-phosphate accumulates and, interestingly, the ability to sporulate is lost (34). A similar accumulation in M. xanthus could conceivably be responsible (indirectly according to model in Fig. 27) for preventing abnormal myxospore formation from occurring during vegetative growth. If this were true, a depletion of L-glycerol-3-phosphate might be necessary for myxospore induction and it is tempting to suggest that glycerol plays a part in such a depletion, because of its similarity in structure to L-glycerol-3-phosphate. This could occur through some type of antagonism between the two three-carbon molecules.

The findings of Sadler and Dworkin (127) seem to rule out a direct antagonism between glycerol and L-glycerol-3-phosphate in M. xanthus

because glycerol is taken up in such small quantities by vegetative cells. Glycerol could act more indirectly to lower endogenous L-glycerol-3-phosphate levels, perhaps by stimulating the glycerol-phosphate dehydrogenase gene of M. xanthus via a membrane surface effect. This would hasten the conversion of L-glycerol-3-phosphate to dihydroxyacetone-phosphate and cause the depletion of accumulated L-glycerol-3-phosphate.

If glycerol-3-phosphate were important in controlling myxospore genes it would be expected that this substance should accumulate during or just prior to germination so that myxospore genes could be turned off. Evidence does not seem to support this, however, and in fact there are indications that a phosphorylated organic substance, perhaps L-glycerol-3-phosphate, is rapidly dephosphorylated during germination. Thus large amounts of inorganic phosphate (1 mM by 10^{10} cells/ml) have been observed to be excreted during the germination of glycerol-induced myxospores in distilled water (25) accompanied by a substantial increase in alkaline phosphatase levels. The phosphatase apparently hydrolyses an endogenous organic phosphate compound such as L-glycerol-3-phosphate and the resultant inorganic phosphate is excreted during germination. A possible explanation of the dephosphorylation is that it is necessary to convert L-glycerol-3-phosphate into a more immediate precursor of the actual repressor of myxospore genes. Since the phosphatase activity of vegetative cells is drastically less than that of germinating cells (26), it would have to be presupposed, for one reason or another, that

adequate levels of the dephosphorylated precursor could be maintained without benefit of high phosphatase levels in vegetative cells.

L-glycerol-3-phosphate may itself be a repressor of myxospore genes, however, it may instead be simply just a precursor of the true repressor. This latter view is in a certain sense more appealing, for if thiamine is the repressor and L-glycerol-3-phosphate just a precursor, the effects of glycerol, the amino acids, the purines and the polyamines could be explained by one unifying mechanism (Fig. 27). All of these effector compounds are either precursors of thiamine or influence thiamine production. The same can not be said for L-glycerol-3-phosphate, many of the effector compounds do not lead directly to L-glycerol-3-phosphate.

Is glycerol-3-phosphate, in fact, a precursor of thiamine? This is an important question to be answered if the unifying model is to be completely believed. One piece of indirect evidence that supports this possibility in bacteria comes from the findings of Newell and Tucker (95) who studied thiamine biosynthesis in S. typhimurium and found that a C_3 compound was involved in the biosynthesis of thiamine (see Fig. 6). The compound was not experimentally identified, but it fits the description of L-glycerol-3-phosphate.

Possible Involvement of Succinyl-CoA in Myxospore Formation

Recent tests have been carried out in the laboratory of Dr. Westby (unpublished data) to ascertain the possible effects of elevated (non vitamin level) concentrations of exogenous thiamine on glycerol-induced

myxospore formation. If thiamine were indeed a repressor of myxospore genes, it was reasoned that elevated levels should prevent myxospore formation (assuming no permeability problem). Unfortunately, the results have been somewhat ambiguous. In the first experimental run the thiamine treated vegetative cells were, as hoped, not converted to myxospores but instead were changed to ovoids and short rods. Subsequent runs, however, did not substantiate the initial results casting doubt on the validity of the first run. Perhaps more disturbing overall than these results are the findings of Lund et al. (81) who discovered that exogenous thiamine actively enhances sporulation in certain Clostridia species. This suggests that thiamine may not be a general repressor of bacterial sporulation.

A possible explanation of the conflicting results on thiamine is that thiamine itself is not the repressor but that instead a thiamine mediated reaction product is the actural repressor. Those substances which trigger myxospore formation and inhibit thiamine synthesis should also inhibit reactions cofactored by thiamine (most likely in thiamine pyrophosphate form) so that it would still be consistent with the main elements of my model if the product of a thiamine-mediated reaction rather than thiamine itself were the true repressor.

Thiamine, in the form of thiamine pyrophosphate, serves as the coenzyme for a number of reactions including: (i) the transketolase reaction (76); (ii) acetyl-CoA formation from pyruvate (76); (iii) succinyl-CoA formation from α-ketoglutaric acid (76); and (iv) several steps in the synthesis of isoleucine, valine, and leucine (152). Since

so many reactions involve thiamine, it would be difficult to pinpoint the exact reaction product that was the hypothesized repressor. Another possible complexity is that all or a number of the reaction products could all be repressors in a theoretically conceivable multivalent system.

If one thiamine mediated reaction out of the many had to be picked out as being the most probable of producing a spore gene repressor, perhaps it would be the reaction from α-ketoglutaric acid to succinyl-CoA. This is the only reaction in the TCA cycle which requires thiamine and the TCA cycle seems to be of such pivotal importance in M. xanthus that it would indeed be strange if it were not somehow involved in myxospore gene repression. The importance of the TCA cycle in M. xanthus can be illustrated if one realizes that it serves as the heart of the metabolism of this organism. All or most catabolic and anabolic pathways in M. xanthus are but arteries of the TCA cycle. The TCA cycle is required in the catabolism of amino acids, which serve as the carbon and energy sources for this organism (extracellular sugars are not catabolized), it is required in gluconeogenesis (156), it is used during glyoxylate pathway metabolism (during myxospore formation) (7, 103) and it is used anabolically to convert required amino acids into dispensable ones.

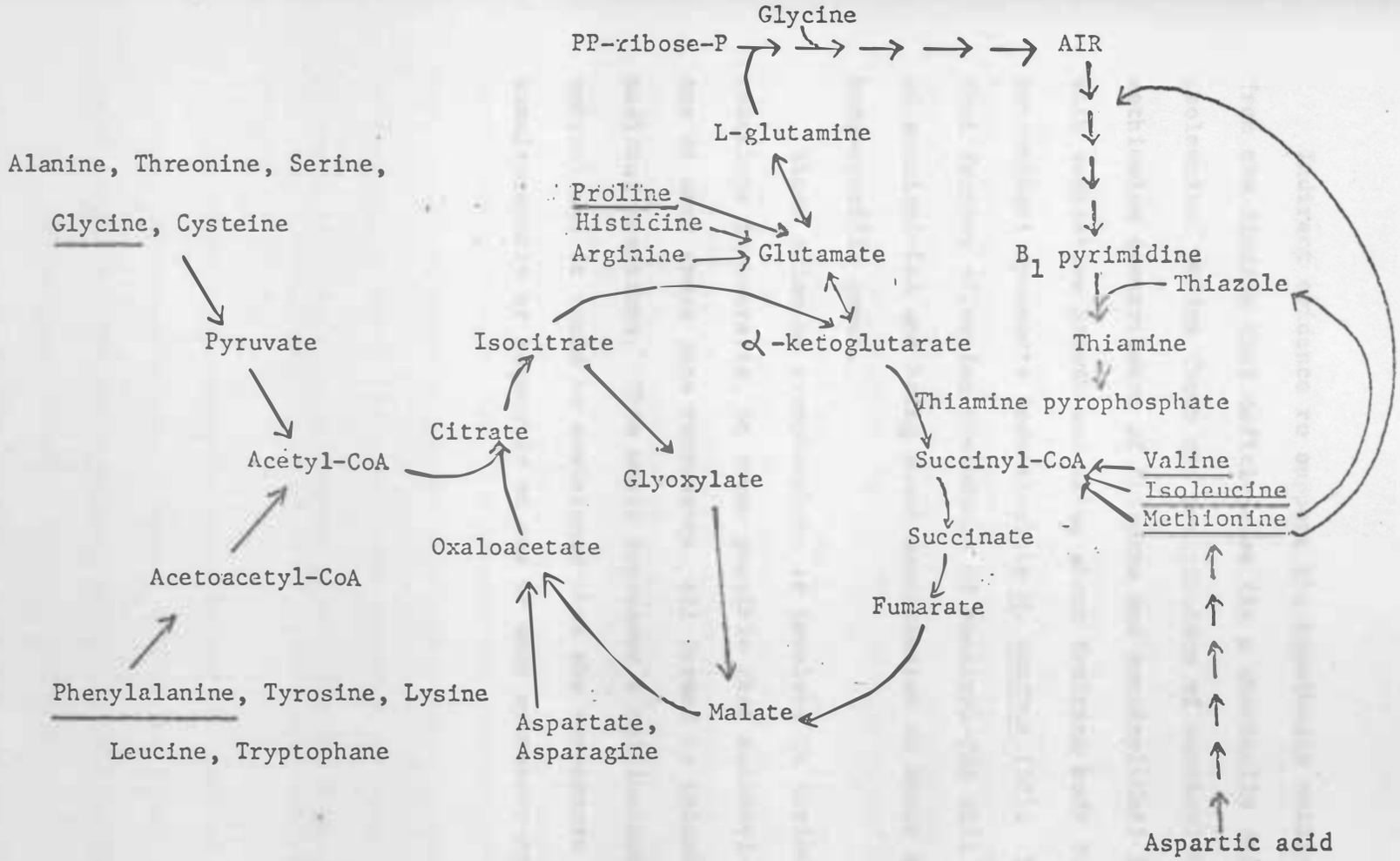
Since succinyl-CoA is a crucial intermediate in the what must be considered important TCA cycle of M. xanthus and since its formation is controlled by thiamine, a likely effector of myxospore formation, it is appealing to propose that succinyl-CoA is a myxospore gene

repressor. Its pivotal place in the metabolism of M. xanthus would place it in the right location to control the myxospore process. The carbon skeletons of many of the required amino acids could be quickly channeled into succinyl-CoA during vegetative growth so that if it were the repressor its levels could be properly maintained to prevent premature myxospore induction (Fig. 28). A depletion of exogenous amino acids, possibly occurring during starvation, coupled with the continued usage of pre-existing succinyl-CoA (via latter part of TCA cycle) could then diminish succinyl-CoA levels, release the repression of myxospore genes and allow for myxospore induction (Fig. 28).

In this scheme, glycerol could be conceived of, as discussed previously, as having its effect through thiamine formation. Hence, glycerol would be pictured as limiting succinyl-CoA formation by depriving the vegetative cell of necessary thiamine (and diminution of pre-existing thiamine could occur through dilution by growth or enzymatic breakdown). This particular model would not require that thiamine be present in above normal levels in vegetative cells (the accumulations of the thiamine-like substance observed on the TL chromatograms would then be hard to account for).

Since certain of the amino acids that are easily channeled into succinyl-CoA are also precursors of thiamine (e.g. methionine and glycine), it is conceivable that their deficiency would lead to an accentuated block of succinyl-CoA formation and perhaps accelerate the conversion of vegetative cells to myxospores.

Fig. 28. Channeling of carbon skeletons of myxospore inducing (underlined) and other amino acids into TCA cycle and thiamine pyrophosphate.


Glycine
PP-ribose-P
AIR
L-glutamine
Alanine, Threonine, Serine,
Glycine, Cysteine
Proline
Histidine
Arginine
Glutamate
B1 pyrimidine
Thiazole
Pyruvate
Isocitrate
α-ketoglutarate
Thiamine
Thiamine pyrophosphate
Citrate
Acetyl-CoA
Glyoxylate
Succinyl-CoA
Valine
Isoleucine
Methionine
Oxaloacetate
Succinate
Acetoacetyl-CoA
Fumarate
Phenylalanine, Tyrosine, Lysine
Leucine, Tryptophane
Aspartate,
Asparagine
Malate
Aspartic acid

Indirect evidence to support the hypothesis outlined above comes from the finding that deficiencies (in a chemically defined medium) in isoleucine, valine (both are contributors of succinyl-CoA) or methionine (contributor of thiamine and succinyl-CoA) levels interfere with vegetative growth and bring about fruiting body formation (and concomitant myxospore induction) in M. xanthus (52). This suggests that factors affecting precursors of succinyl-CoA will lower the level of succinyl-CoA and bring about derepression of spore and/or fruiting body-specific genes.

Since thiamine pyrophosphate is involved in various metabolic reactions in bacteria, it seems possible that succinyl-CoA may be only one of many spore gene repressors, all formed by thiamine pyrophosphate-mediated reactions. This would represent a multivalent system of control and it could be envisioned that the repressors could act simultaneously or sequently on one or many myxospore-specific genes.

CONCLUSIONS

1. *In vitro* studies indicate the presence of both PP-ribose-P amidotransferase (EC 2.4.2.14) and ribose-5-phosphate aminotransferase (no EC number) in vegetative and myxospore (2.5 h and 8 h) extracts of *M. xanthus* CW-2. Both enzymes appear to be present at about the same level (per milligram of protein) in vegetative cells, myxospores (2.5 h and 8 h), and in a bacterial prototype, *S. typhimurium*.

2. The dose response of the CW-2 vegetative and myxospore amido- and amino-transferases towards AMP and GMP suggests that the allosteric structure of both enzymes is changed little by sporulation.

3. Both the CW-2 amido- and amino-transferases were inhibited to varying degrees by a variety of purine nucleotides besides AMP, GMP, and 3':5' cyclic AMP.

4. Glycine, adenine and guanine cellular uptake studies indicate that glycerol-induced CW-1 8 h myxospores, although supposedly dormant, take up all of these compounds at a rate similar or greater than that of vegetative cells. Each of these compounds is taken up by the different cell types at a characteristic rate to a characteristic saturation level.

5. Glycine uptake was reduced during the initial stages of glycerol induced myxospore formation (first hour) but increased during later stages and was above vegetative cell levels during germination.

6. Purine uptake studies suggest the presence of interconversion and salvage routes in the different cell types of *M. xanthus* CW-1.

Adenine was taken up in a relatively linear fashion whereas guanine was taken up in step-like increments.

7. Germinating myxospores of M. xanthus CW-1 take up external glycine, adenine, and guanine more rapidly than any other cell types. This could be a reflection of the faster growth rate of germinating myxospores compared to vegetative cells.

8. CW-1 vegetative cells, myxospores and germinating cells partial repressed of purine de novo synthesis take up glycine somewhat more rapidly than fully repressed cells. This suggests that purine de novo synthesis can occur continuously during the life cycle (glycerol induction) of M. xanthus (provided cells are partially derepressed of purine nucleotide synthesis).

9. CW-1 vegetative cells, myxospores and germinating cells (partially repressed) convert glycine-2-^{14}C in vivo into ^{14}C-ATP and -GTP. This suggests, more strongly, that purine de novo synthesis can occur continuously during the life cycle of M. xanthus.

10. A thiamine-like substance was found in certain cell extracts and may be related to myxospore induction.

11. No evidence could be found for the presence of HPN or MS in M. xanthus cell extracts.

12. A model for the thiamine or succinyl-CoA control of myxospore formation in M. xanthus was presented. This model proposes that a thiamine substance or succinyl-CoA may be a catabolite repressor or corepressor of myxospore (and fruiting-body) formation.

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